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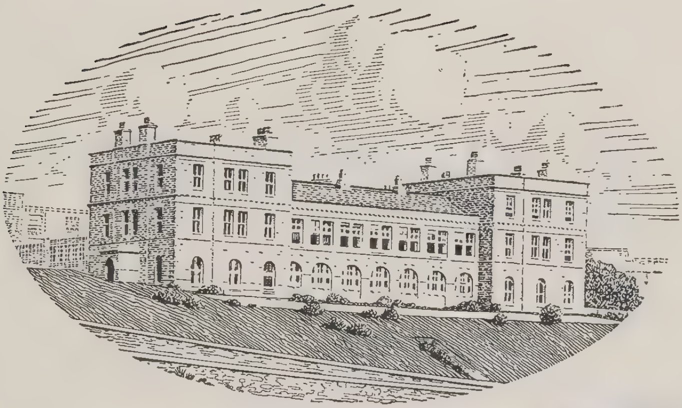
JOURNAL OF THE
MARINE BIOLOGICAL ASSOCIATION
OF THE UNITED KINGDOM

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)
and published by the Cambridge University Press
Cambridge, and Bentley House, London

Agents for Canada and India: Macmillan

JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

1685⁶⁰



THE PLYMOUTH LABORATORY

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VOLUME 33

1954

CAMBRIDGE
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1954

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CORRIGENDA

Vol. 30

Page 639. Section xvi D, 1. For *Micr.* read *Phil.*

Vol. 33

Page 241. Line 12. *For* tracheae and cells *read* tracheae end-cells.
598. Fig. 4, top graph. *For* Vitamin A (i.u./g) *and* Astaxanthin (μg/g) *read* Vitamin A (i.u./specimen) *and* Astaxanthin (μg/specimen).
790. Address of Fisher, L. R. *For* Bucks. *read* Berks.
797. Line 2. After *University,* insert *Montreal,*.

J. Mar. biol. Ass. U.K. (1954) 33, 1–19
Printed in Great Britain

THE EFFECTS OF WAVE-ACTION ON THE DISTRIBUTION AND NUMBERS OF THE COMMONER PLANTS AND ANIMALS LIVING ON THE PLYMOUTH BREAKWATER

By A. J. Southward and the late J. H. Orton, F.R.S.

(Text-figs. 1–7)

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INTRODUCTION

The breakwater in Plymouth Sound offers a simple case of two adjacent populations of intertidal organisms subjected to quite different degrees of exposure to wave-action. The other environmental factors, with a few exceptions, are approximately the same each side, and the effects of the wave-action should be clearly demonstrable.

Previous experience in the Isle of Man (Southward, 1953) has proved that evidence of variation in the numbers of the plants and animals is as important as variation in their zonation, and that the numbers may, in fact, show the greater changes in relation to differing degrees of exposure to wave-action. The work was planned on a quantitative basis, and the breakwater proved a fortunate choice, since it offers relatively uniform slopes throughout the greater part of the intertidal zone. The latter factor is almost a necessity for a quantitative study, but a quality lacking in most of the rocks exposed in the Plymouth area.

The investigation was suggested by Prof. Orton, and was planned and begun as a joint study with the aid of a grant from the E. T. Browne Fund of the Royal Society. Various factors intervened, not the least of which were delays due to inclement weather, and most of the field work was carried out by

A. J. S. alone, in September 1951. This account was prepared by A. J. S. and revised by Prof. Orton shortly before his death.

We are indebted to Mr F. S. Russell, F.R.S., and to various members of the staff of the Marine Biological Association for assistance, and to Dr R. G. Evans for helpful criticism of the typescript.

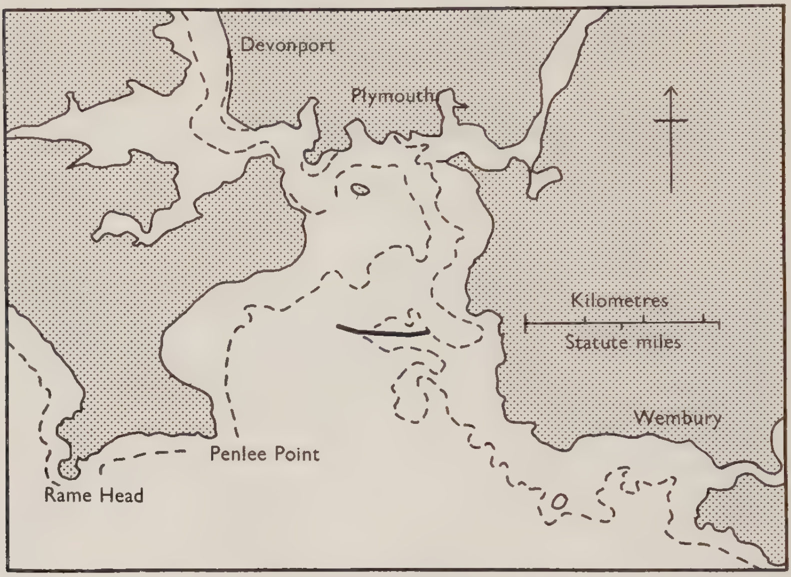

Fig. 1. Map of the Plymouth area showing the position of the breakwater. — —, approx. 5 fathom (9 m.) line.

The Environment

The general environment of the Plymouth area has been discussed by Orton (1920), Colman (1933) and Evans (1947*b*), and the main features of the breakwater have been described by Lysaght (1941). It is therefore only necessary to mention certain factors of importance to this study.

Substratum. The main constituent of the breakwater is a local limestone, and the northern side, down to low-water neap level, consists entirely of blocks of this material cemented together. From low-water neaps down, however, the north side consists of limestone and cement rubble of varying size: some of the larger masses may project as high as mid-tide level (Fig. 2).

On the southern side of the breakwater, the sharp ledges at each end (Fig. 2, sections *A* and *E*), the ledge at low-water neaps (sections *C* and *D*) and various other isolated parts (particularly on section *B*) are faced with granite. The granite frequently stands proud of the limestone by as much as 5 cm., indicating the extent to which the latter has eroded, and in places, especially towards the western end, this erosion has produced a somewhat irregular

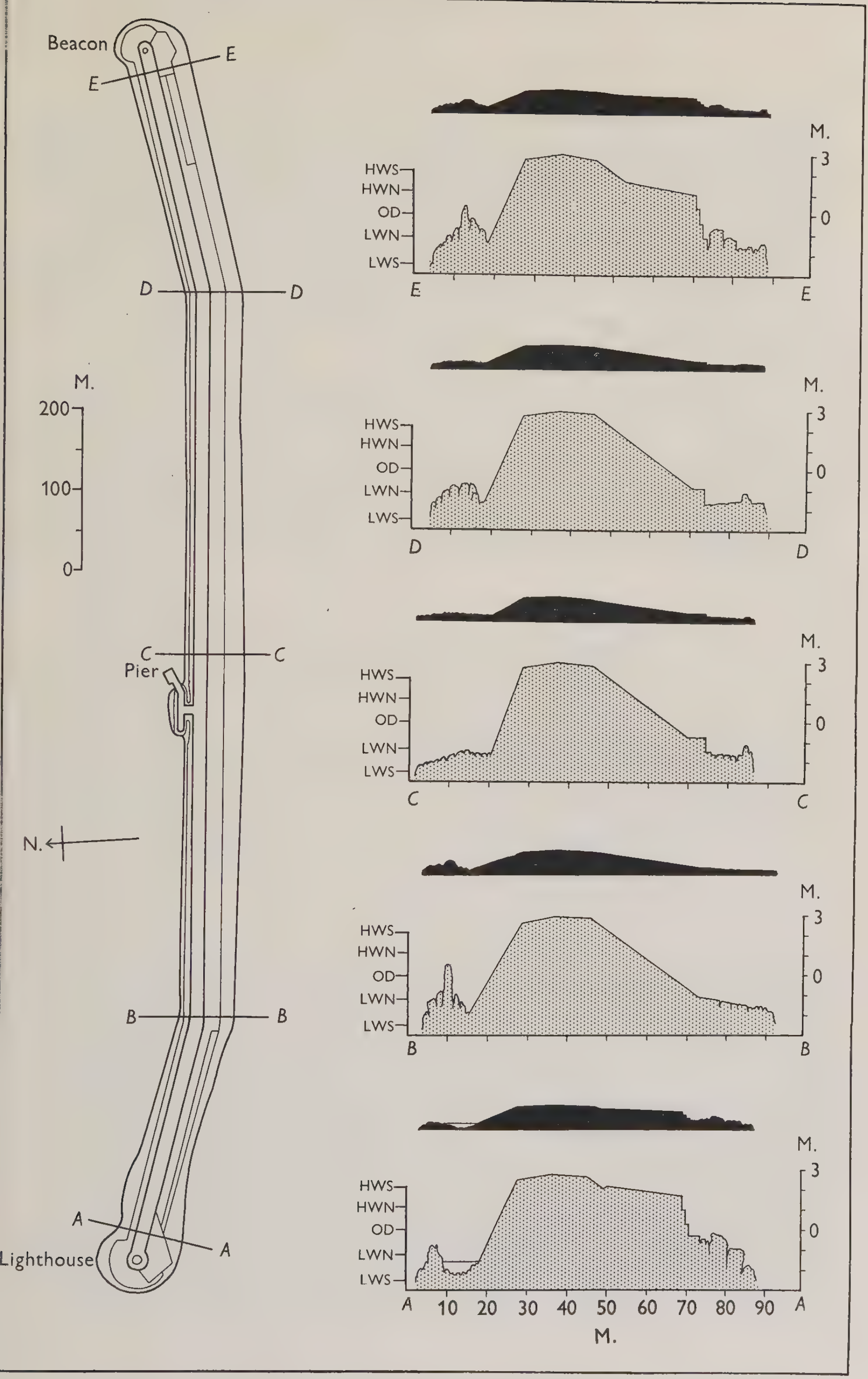
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Fig. 2. Plan of the breakwater showing the traverses (*A–A* to *E–E*) and sections across them. The sections in black show the true profiles, while those stippled have their vertical scale exaggerated (five times the horizontal scale) to bring out the salient features.

surface, with pools up to several centimetres deep. If the blocks on the south side were ever cemented together, little trace of it remains, and there are deep crevices a few centimetres wide and several centimetres deep, between them. Below low-water neaps on the south side there is a gently sloping layer of roughly squared limestone blocks, covered with concrete in places, and at the outer edge of this is a double row of large concrete blocks, approximately 2 m. high. At each end of the breakwater (Fig. 2, sections *A* and *E*) the space between these blocks and the main body of the breakwater is filled with limestone rubble and rough concrete blocks.

Orientation. The breakwater runs roughly east–west (Fig. 1), and the north-facing side has a slope of from 30 to 35° from the horizontal, while the south face is only 10° from the horizontal. As a consequence, the sunlight that reaches the north face during the middle part of the day will be more oblique than that on the south face. Thus, although the north face cannot be regarded as being in the shade, there is a possibility that some of the adverse effects of the occurrence of low-water springs at midday (see Orton, 1920) may be mitigated.

METHODS

Details of the wave-action observations are given in a separate section (p. 5).

The dimensions of the breakwater are such that the methods used previously ('grid surveys', see Southward, 1953) would be most impractical, and for the investigations on the plants and animals a series of five traverses was marked out across the breakwater. The positions of the traverses and cross-sections at each traverse are shown in Fig. 2. Levels were transferred from a recently surveyed tide-staff on the breakwater fort to the breakwater pier, by means of the water's edge on a dead calm day. Fixed marks at the highest points of the traverses were then 'levelled' in a closed circuit from the pier, the error being distributed over all the readings. The individual traverses were levelled from the fixed marks, and as the number of sightings was small, the error could be ignored.

The eastern end of the breakwater was found to be 0·3 m. higher than the western end, the relative heights being 3·18 and 2·88 m. above Ordnance Datum (Liverpool). All heights are given in metres above or below O.D. (Liverpool), which, although not true mean sea level, is at Plymouth a better approximation to mean tide level than the more recently established O.D. (Newlyn). The two levels, M.T.L. and O.D. (Liverpool) differ by only 0·07 m., and for the present purposes can be taken to be the same (see Table I).

On each traverse notes were made of the upper and lower limits of the commoner organisms. Quantitative observations were made at each 0·5 m. above or below M.T.L., the percentage cover of the plants being first estimated with the aid of a wire frame of 1 × 1 m. divided up into a hundred decimetre squares. The barnacles and littorinids were then counted on several decimetre squares, or the whole metre square if sparse, and finally the limpets and top-shells were removed from the whole or half of the metre square for sorting. The length of the limpet shells was later measured to the nearest millimetre.

Owing to the broken nature of the region at L.W.N. on the north side the quantitative observations were not completed on certain traverses. Traverse *A* north side ended in a pool, and was not carried below M.L.W.N.

The animals and plants in the deep crevices between the blocks and in the pools were not included in the survey.

TABLE I. TIDE LEVELS FOR PLYMOUTH, BASED ON THE ADMIRALTY TIDE TABLES (1951)

Levels	Abbreviations used in text	Height relative to C.D. (Devonport) Feet	Height relative to C.D. (Devonport) Metres	Height relative to O.D. (Liverpool) Feet	Height relative to O.D. (Liverpool) Metres	Height relative to O.D. (Newlyn) Feet	Height relative to O.D. (Newlyn) Metres
Extreme high-water springs	E.H.W.S.	17·0	5·2	8·5	2·6	8·0	2·4
Mean high-water springs	M.H.W.S.	15·70	4·79	7·28	2·22	6·78	2·07
Mean high-water neaps	M.H.W.N.	12·25	3·72	3·83	1·15	3·33	1·00
Ordnance datum (Newlyn)	O.D.	8·92	2·72	0·50	0·15	0	0
Ordnance datum (Liverpool)	O.D.	8·42	2·57	0	0	−0·50	−0·15
Mean tide level	M.T.L.	8·20	2·50	−0·22	−0·07	−0·72	−0·22
Mean low-water neaps	M.L.W.N.	4·60	1·40	−3·82	−1·16	−4·32	−1·31
Mean low-water springs	M.L.W.S.	0·18	0·06	−8·24	−2·51	−8·74	−2·66
Chart datum	C.D.	0	0	−8·42	−2·57	−8·92	−2·72
Extreme low-water springs	E.L.W.S.	−1·6	−0·5	−10·0	−3·0	−10·5	−3·3

WAVE-ACTION

For the purpose of the present study, significant wave-action was defined as the occurrence of waves washing over the top of the breakwater at, or before, high water. With the tide at M.H.W.S. this requires a minimum height of wash of 0·6 m. above the true tide height at the western end of the breakwater, but at neap tides, of course, much greater wash is required. However, during rough weather the observations had to be made from the land (with the aid of field-glasses), as it was impossible to reach the breakwater, and no other criterion of wave-action was feasible over the distance involved (3·5 km.). During a period of 28 days' observations it was found that practically all winds with a velocity of 10 m.p.h. (16 km./hr.) or more, blowing from the sector W.–S.–S.E. were associated with waves breaking over the top of the breakwater. Some of the more extreme examples of the observations are shown in Table II: nearly all of these were recorded from the shelter of the laboratory.

By applying the the results of the observations to wind records it is possible to produce an approximate exposure index. From Table III, columns 1 and 2, the values for 1950 and 1951 were 38 and 32% respectively. These figures are similar to those arrived at by Lysaght (1941), who, however, considered the sector S.W.–S.–S.E. to be critical (Table III, column 3).

TABLE II. SOME OF THE MORE EXTREME EXAMPLES OF WAVE-ACTION ON THE BREAKWATER

Date (1951)	Wind direction and velocity (m.p.h.) at 09.00 hr.	Maximum wind velocity (gusts in m.p.h.) during succeeding 24 hr.	West end			East end		
			Time of waves starting to wash over	Predicted height of tide at the time (metres relative to O.D.)	Calculated minimum height of wash (m.)	Time of waves starting to wash over	Calculated height of tide at the time (metres relative to O.D.)	Calcula minimu height wash (
26 Aug.	W.S.W. 16	42	09.30	0	2·8	No wash, even at high water		
27 Aug.	S.W. 18	35	11.00	0·4	2·3	No wash, even at high water		
28 Aug.	S.S.W. 23	48	11.45	0·3	2·4	13.00	1·3	1·7
29 Aug.	S.S.W. 20	44	12.00	−0·2	3·0	15.00	1·6	1·4
13 Sept.	S.S.W. 41	57	11.45	−1·3	4·1	12.30	−0·6	3·6
14 Sept.	S.S.W. 19	52	13.50	−0·2	3·0	No observation		

TABLE III. DISTRIBUTION OF WINDS AT PLYMOUTH

Month	Percentage days of wind blowing from the sector W.–S.–S.E. with a velocity of 10 m.p.h. or more		Percentage of winds (all velocities) blowing from the sector S.W.–S.–S.E., for the period 1893–1922 (from Lysaght, 1941, p. 43)
	1950	1951	
Jan.	32	29	33
Feb.	64	39	34
Mar.	45	42	32
April	30	27	30
May	16	16	35
June	47	40	38
July	35	10	36
Aug.	68	55	40
Sept.	43	43	30
Oct.	39	6	32
Nov.	20	40	26
Dec.	16	35	35
Year	38	32	33

All these exposure indices seem low compared with similarly derived indices for other places (e.g. 70% at Port St Mary, Isle of Man, see Southward, 1953), but there is no evidence that the breakwater is any less liable to wave-action. In spite of the wind and wave records there were only 3 days out of the 28 without any wave-action on the south side, and it would seem that refracted waves and swells play a much bigger part in the wave-action on the breakwater than at Port St Mary. There can be no precise comparison of the two places without long-period wave recordings.

In comparing the wave-action on the north and south faces of the breakwater we are on a firmer basis. It has already been pointed out that, during the period of wave-action observations, there were only 3 days when there was no appreciable wave-action on the south side; in the remaining 25 days the wave-action produced a wash of at least 0·3 m. above true tide height, if not more. On the north face, however, even during north-easterly winds reaching gale force at times, the wash was not seen to exceed 0·3 m., which is less than a tenth of the maximum wash observed on the south side.

It must be noted that, although the north face would appear to be almost completely sheltered from the mechanical effects of wave-action, it is wetted by wash when waves break over the top of the breakwater from the south side. This may accentuate the possible effects (noted on p. 4) of the obliquity of the sunlight received by the north face.

From the topography it might be expected that the eastern end of the breakwater would experience greater wave-action than the western end, which appears to be sheltered, to some extent, by Rame Head and Penlee Point (Fig. 1). However, this was not the case, and the observations show that there was considerably more wave-action on the western end than on the eastern end. The eastern end is only 0·3 m. higher than the western end, yet, on several occasions it was observed that the waves did not begin to wash over it until the tide had risen by from 0·5 to 1·6 m. above the level at which it stood when waves began washing over the western end (see Table II). On two occasions waves began washing over the western end more than 2 hr. before high water, but there was no wash at the eastern end even at high water.

The evidence thus indicates that the wave-action at the western end may be some 10–40% greater than at the eastern end of the breakwater. It is possible that the deeper water at the western end of the breakwater, which stands in 12–15 m. of water at low tide, may allow the closer approach of larger waves, with consequently greater wash on breaking. The eastern end stands in only 6–9 m. of water at low tide, and may be sheltered by the shoals to the south (Fig. 1).

PLANTS

At first impression there seemed to be a marked difference between the two sides of the breakwater, the south side appearing to be practically bare of algae, while the north face bore a comparatively dense population. In general, further investigation confirmed this view, but the population on the south side was greater than expected.

The species dealt with are described below: their nomenclature follows that of Newton (1931). The calcareous algae of the genera *Lithothamnion*, *Lithophyllum* and *Melobesia* were not identified and are referred to collectively as ‘lithothamnia’.

Enteromorpha sp. Slight patches of *Enteromorpha* mixed with filamentous green algae occurred on the northern slope between M.H.W.N. and M.T.L. These patches were commonest towards the western end (traverses *A* and *B*) but did not exceed 20% cover. On the south side *Enteromorpha* was present all along the ledge just above M.L.W.N., but was commonest between traverses *C* and *D*; many of the limpets had a dense growth on their shells.

Laminaria saccharina and *Saccorhiza polyschides* (*bulbosa*) had similar distributions, at, and below, M.L.W.S. all along the northern side: on the south side they were restricted to the larger crevices between the rough blocks below L.W.N., occurring as high as 0·5 m. above M.L.W.S. This distribution confirms previous evidence (Evans, 1947*b*) that these species are generally restricted to places sheltered from wave-action. However, *Laminaria saccharina* seems to require a certain amount of water movement.

L. digitata. While some young plants of this species occurred as high as M.L.W.N. among the *Himanthalia* on the south side, the main upper limit, defined here as the zone of 50% or more cover, was a trifle higher on the north face, where it began at 0·4 m. above M.L.W.S. compared with M.L.W.S. on the south side. To some extent, this bears out a tendency, noted by Evans (1947*b*) and Southward (1953), for the main upper limit of *Laminaria* on steep or broken shores to be higher in sheltered places. However, on both sides of the breakwater there was practically 100% cover below M.L.W.S.

Fucus spiralis. As shown in Fig. 3, this species was well represented on the north side, where it occurred from M.H.W.S. down to M.T.L. The maximum cover was found at M.H.W.N. and just above, increasing from 20% on traverse *A* to over 50% on traverse *E*. It was poorly represented on the south side, only a few tufts being found on traverse *A*, and nowhere exceeded 6% cover. The zone varied quite widely on the south side but was always narrower than that on the north side. It is worth noting that on the north side of traverses *A*, *B* and *C*, the lowermost plants occurred side by side with the uppermost plants of *F. serratus*. This overlapping of the two species is not recorded by Colman (1933) or Evans (1947*b*), and has not been noticed in the Isle of Man, but may occur in Cardigan Bay (Evans, 1947*a*). It is possibly due to an unusually low lower limit of the *spiralis*, which itself may be due to the absence of *Ascophyllum*.

Fucus vesiculosus (Fig. 3). As might be expected from the known tolerance by this species of considerable wave-action (Kitching, 1935; Evans, 1947*b*; Southward, 1953), the differences between the populations on the north and south sides of the breakwater were much less than those of the other species of *Fucus*. In general, the cover was lower on the south side where it reached a maximum of 14% compared with up to 26% on the north side, but on traverse *A* the cover on either side did not exceed 2%. The upper and lower limits varied widely, the narrowest zone being found on the north side of traverse *A*, from 0·4 m. above M.T.L. to M.L.W.N. In other places, such as the south side of traverse *B*, and the north side of traverse *E*, the species occurred up to M.H.W.N.

F. serratus (Fig. 3). This species is usually found higher up the shore in sheltered situations (Evans, 1947*b*; Southward, 1953). On the breakwater, the main upper limit on the north side was at approximately M.T.L., compared with about M.L.W.N. on the south side. With the exception of traverse *B*, the population was denser on the north side, where it exceeded 90% cover in places, compared with a maximum of 40% on the south side.

Ascophyllum nodosum was not found.

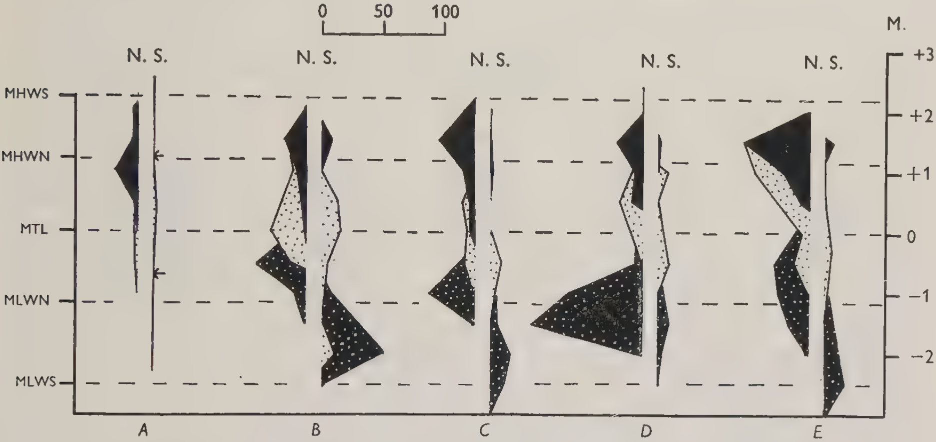

Fig. 3. The distribution of three species of *Fucus* on the breakwater. On the south side of traverse *A* the limits between the species are marked by arrows; on the north side of *A*, *F. serratus* could not be investigated and is not shown. On this, and subsequent diagrams, frequency polygons for the north and south sides of the traverses are indicated by the letters N. and S. respectively. ■, *F. spiralis*; ▤, *F. vesiculosus*; ▩, *F. serratus*. The scale denotes percentage cover.

Pelvetia canaliculata was not observed on any of the traverses other than the north side of *C*, where it occupied a narrow zone from 0·8 m. above M.H.W.N. to 0·3 m. above M.H.W.N., and reached 36% cover. Traverse *C* is very near the pier (see Fig. 2) and at high water is probably the most sheltered region of the whole breakwater. A few isolated tufts of *Pelvetia* were seen on the south side, between traverses *B* and *C*.

Himanthalia lorea formed a well-defined belt on both sides of the breakwater, from about 0·5 m. above M.L.W.N. down to almost M.L.W.S. On both sides it achieved 100% cover in places, but the region of maximum cover was higher on the north side, at approximately M.L.W.N., than on the south side, at 0·8 m. below this level. The species was least developed on the south side of traverse *A*, where it did not exceed 12% cover.

Porphyra umbilicalis. A few plants of this species, presumably the remnants of the settlement in the autumn of the previous year, were present all along the south side between M.H.W.S. and M.H.W.N., and at M.L.W.N. They were commonest just above M.H.W.N., where they reached 5% cover. A few tufts also occurred on the north side of traverse *A* at M.H.W.N.

'Lithothamnia'. The upper limit of the 'lithothamnia' and the associated *Corallina*, excluding pools and wet places, usually corresponds to the lower limit of the barnacles: on steep slopes or broken shores these limits are usually lower in the more wave-beaten places (Southward, 1953). This was the case on the breakwater, the lowest position of the limits being found on the south side of traverse *A*, at 0·5 m. below M.L.W.N., compared with at M.L.W.N. on the south side of traverses *C*, *D* and *E*. On the north side the limits were affected by the other algae present, especially *Fucus serratus*, beneath the cover of which the 'lithothamnia' occurred up to M.T.L., but was otherwise approximately 0·5 m. below M.T.L.

Rhodymenia palmata occurred on all traverses, with an upper limit between M.T.L. and 0·5 m. below. Surprisingly, the maximum densities were found both on the south side (20% cover on traverse *A*) and on the north side (20% cover on traverse *E*). In the latter situation the species was associated with, and partly under, *Fucus serratus*, but in the former it was largely present on the bare rock. Presumably the more wave-beaten situation (*A*) provides as much protection from drying as does that beneath *Fucus*.

Lichina pygmaea is generally commonest in exposed places (Evans, 1947*b*). On the breakwater it occurred only on the south side, but was quite sparse, not exceeding 4% cover. The zone, from E.H.W.S. to 0·3 m. above M.H.W.N., is narrow compared with other places in the Plymouth area where it may extend to M.T.L. (Colman, 1933; Evans, 1947*b*). The sparseness and restricted zone suggest that the breakwater is an unsuitable habitat for the species, possibly due to the smooth nature of the limestone blocks.

ANIMALS

The nomenclature of the animals is based on that of the *Plymouth Marine Fauna* (Marine Biological Association, 1931), with the following exceptions: *Patella intermedia* Jeffreys 1865 = *P. depressa* Pennant 1777 (see Fischer-Piette, 1938, 1948); *P. aspera* Lamarck 1819 = *P. athletica* Bean 1844 (see Fischer-Piette, 1938, 1948); *Littorina saxatilis* Olivi 1792 = *L. rudis* Maton 1797 (see Colman, 1932).

Chthamalus stellatus. On all but the gentlest of slopes this species would appear to show the same relation to wave-action as does *Balanus balanoides*, i.e. an increase in exposure raises the upper limit, lowers the lower limit, and increases the population density (Hatton & Fischer-Piette, 1932; Moore, 1935; Southward, 1953). On the breakwater this was true of the southern face as compared with the northern face, but the difference in number was much more noticeable than the differences in the upper and lower limits (Fig. 4). Thus, the upper limit on the north side was at approximately E.H.W.S. (0·3 m. above M.H.W.S.). On the south side the species did not occur any higher than the general upper surface of the breakwater (0·6 m. above E.H.W.S.), in spite of the numerous structures, such as shelters and blockhouses, which extended above this height. The lower limit on the south side varied from M.L.W.N. at the eastern end to 0·4 m. below this level at the western end. This is some 0·7–1·1 m. below the lower limit on the northern side, which was roughly 0·5 m. above M.L.W.N. (see Fig. 4).

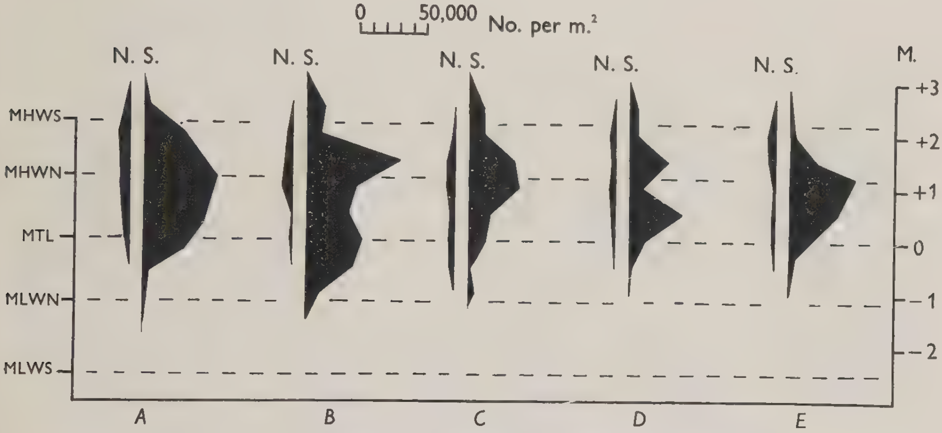

Fig. 4. The distribution of *Chthamalus stellatus* on the breakwater.

The counts showed much greater differences between the two sides of the breakwater. On each traverse, at the region of maximum abundance (about M.H.W.N.), the numbers of *Chthamalus* on the south side were over five times as great as those on the north side. The greatest number found on the north side was a mere 8000/m.², compared with a maximum of 68,000/m.² found on the south side. However, the latter figure refers to a granite substratum: on limestone the numbers did not exceed 40,000/m.², and were always lower than on the nearby granite. This difference in density of population between the two types of rock seems to be a fairly general phenomenon, and in this case may be related to the rougher surface of the granite.

It must be noted that on both sides of the breakwater the barnacle population was greater at the western end, although this was most marked on the south side (Fig. 4).

Balanus balanoides. At the time of the field work this barnacle had practically disappeared from the Plymouth area (Southward & Crisp, 1952). Isolated specimens were found on the south side of the breakwater, between M.T.L.

and M.L.W.N. on traverses *A*, *B* and *C*. The numbers did not exceed 2/m.^{2}, and the distribution was very erratic.

B. perforatus was found on both sides of the breakwater, between 0·8 m. above, and 0·8 m. below, M.L.W.N. The numbers were somewhat greater on the south side, with a maximum of 16/m.^{2}, compared with up to 6/m.^{2} on the north side.

B. crenatus occurred on the south side from 0·8 m. below M.L.W.N. to the lowest level investigated (E.L.W.S.), and was commonest towards the western end where it reached a density of up to 40/m.^{2} on traverse *A*. On the north side it was noticed only on the piles of the pier.

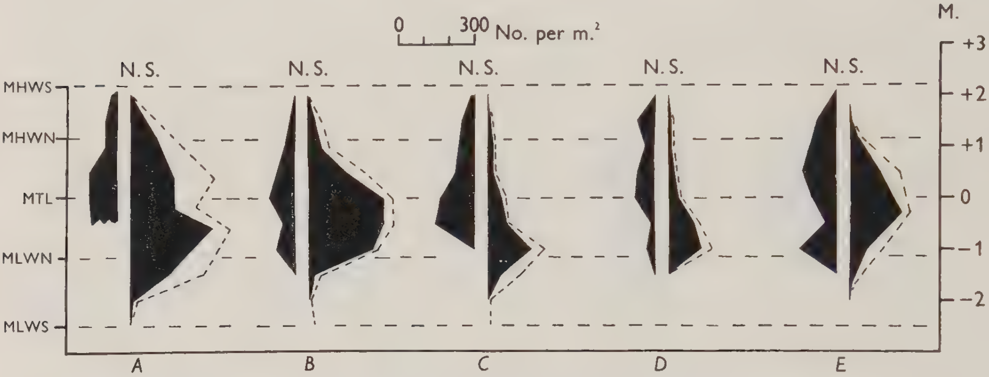

Fig. 5. The distribution of *Patella vulgata* (black) and total *Patella* (broken line) on the breakwater.

Patella vulgata (Fig. 5). The upper limit of *P. vulgata* was found to be practically the same on both sides of the breakwater, at 0·1–0·2 m. below M.H.W.S. According to previous evidence (Orton, 1929; Evans, 1947*b*; Southward, 1953) this species is not normally found above M.H.W.N., except on north-facing shaded surfaces, or on rocks receiving considerable wave-action. It would therefore appear that, on the breakwater, the effect of wave-action on the south side is more or less balanced by the effect of the greater shade from the sun on the north side. The moderately dense growth of *Fucus spiralis* on the north side up to M.H.W.S. may help to maintain the upper limit of *Patella* on that side by offering further protection from desiccation.

The lower limits differed by about 0·5 m.: on the north side the limit varied from 0·2 m. above, to 0·3 m. below, M.L.W.N., while on the south side it varied from 0·3 m. below M.L.W.N., to M.L.W.S. The occurrence of the lowest lower limit at the western end of the south side supports the view that this difference is due to the wave-action (cf. Southward, 1953), but it is possible that the higher lower limit on the north side is more a result of the layer of silt present on the rocks below L.W.N. rather than to the shelter from wave-action itself.

From Fig. 5 it will be seen that the largest numbers of *P. vulgata* occurred on the south side of each traverse, reaching a maximum towards the western end of the breakwater. In general, this agrees with findings in the Isle of Man, where, except on very steep slopes, the numbers tended to increase with increase in exposure to wave-action (Southward, 1953). However, the differences between the two sides of the breakwater were not very marked, especially on purely limestone substrata. For example, on traverses *C* and *D* the greatest numbers found on the north side were 134 and 76/m.^{2} respectively, compared with 156 and 130/m.^{2} on the south side. Above M.T.L., in fact, the north-side population was the greater. On the granite substrata of traverse *A*, however, the numbers reached 330/m.^{2} just below M.T.L., practically twice the population at the same level on the limestone rock, and nearly three times that at the same level on the north side.

The problem of population density in *Patella* is quite inseparable from that of size, since there is usually a broad inverse correlation between them (Jones, 1948; Southward, 1953). The smaller sizes present in wave-beaten situations (Fischer-Piette, 1941, 1948; Evans, 1947*b*) may therefore be due to the greater numbers present in such places (Southward, 1953). On the breakwater the limpets tended to be larger on the north side, i.e. there was a bigger proportion of the larger size-groups (40–70 mm. in length), and Fig. 6 shows a representative series of histograms for the M.T.L. samples. The size difference was most marked at, and above, M.T.L., the proportion of larger *Patella* on the south side increasing towards L.W.N. The absence of larger limpets was particularly noticeable on the densely inhabited granite substrata on the south side (Fig. 6*A*, *B*).

The restriction of *P. intermedia* and *P. aspera* to the more wave-beaten places (Fischer-Piette, 1948; Evans, 1947*a*, *b*) is clearly demonstrated by the limpet samples from the breakwater. On the north side, out of about 2000 specimens examined, only ten specimens of *P. intermedia* and six *P. aspera* were found, and these nowhere exceeded a density of 4/m.^{2}. On the south side these species were present on all traverses, and at certain levels constituted almost half of the total limpet population (see Fig. 5).

P. intermedia. The upper limit of this species was a little below that of *P. vulgata*, varying from 0·2 m. below M.H.W.S. at the western (more exposed) end, to about M.H.W.N. at the eastern end of the south side. The lower limit on the south side was at about M.L.W.N., except on traverse *A*, where the species occurred to 0·3 m. below this. The few specimens that occurred on the north side were found between M.H.W.N. and M.T.L. On the south side the greatest numbers were found around M.T.L., reaching a maximum of 160/m.^{2} on traverse *A*. The density was generally higher on the granite than on the limestone.

P. aspera. On the south side, the upper limit was higher at the western end, where it occurred at M.H.W.N. on traverses *A* and *B*, compared with M.T.L. on traverse *D*. On traverses *A*, *B* and *C* the species was present down to M.L.W.S. and possibly below, but on *D* it did not occur more than 0·3 m. below M.L.W.N. Of the few specimens found on the north side, one occurred at M.T.L., the others at M.L.W.N. The numbers on the south side were generally greater on the limestone than on the granite, reaching a maximum of 80/m.^{2} on traverse *C*, just below M.L.W.N.

Gibbula cineraria was the least common of the three species of top-shells found on the breakwater. It occurred only once in the samples, at M.L.W.N. on the south side of traverse *B*, but it seems possible that it may have been present on the north side below the limit investigated (M.L.W.S.).

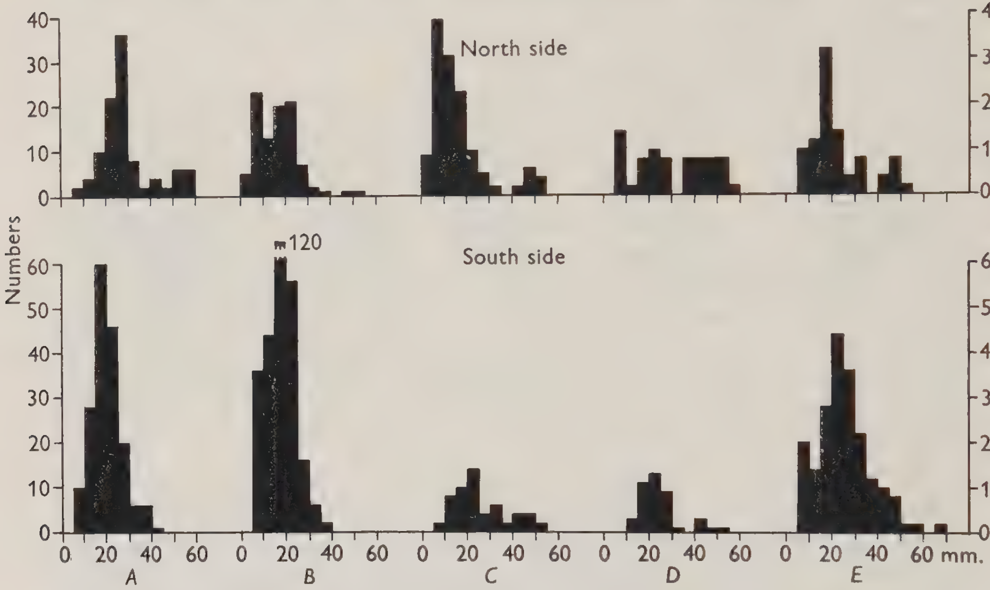

Fig. 6. Frequency distributions, in 5 mm. groups, of 1 m.^{2} samples of *Patella vulgata* taken at M.T.L. on each traverse on the breakwater.

G. umbilicalis was the commonest top-shell, but, except for one specimen in a pool at M.H.W.N. on the south side of traverse *D*, it was confined to the north side. It was absent from traverse *A*, sparse on *B*, but commoner on *D* and *E*, where it reached a density of 15/m.^{2}, and ranged from M.H.W.N. to M.L.W.N. In other places in the Plymouth area it has been found down to M.L.W.S. (Evans, 1947*b*).

Osilinus lineatus occurred only on the north side of traverse *E*, between M.H.W.S. and M.H.W.N.

Littorina neritoides. The distribution of this species on the breakwater has been described by Lysaght (1941). In the present work the observations were confined to the quantitative samples on the traverses, and the specimens which occur in crevices and pools were ignored. Thus the numbers found in 1951 were generally less than those found by Lysaght.

When the crevice-living specimens were omitted it was found that the remaining specimens were associated with *Chthamalus*, occurring between the shells or inside dead ones. The distribution thus followed that of *Chthamalus* quite closely (Figs. 4, 7). However, there were a number of differences: the numbers were no higher on granite than on limestone, and the lower limit was approximately 0·5 m. higher than that of *Chthamalus*.

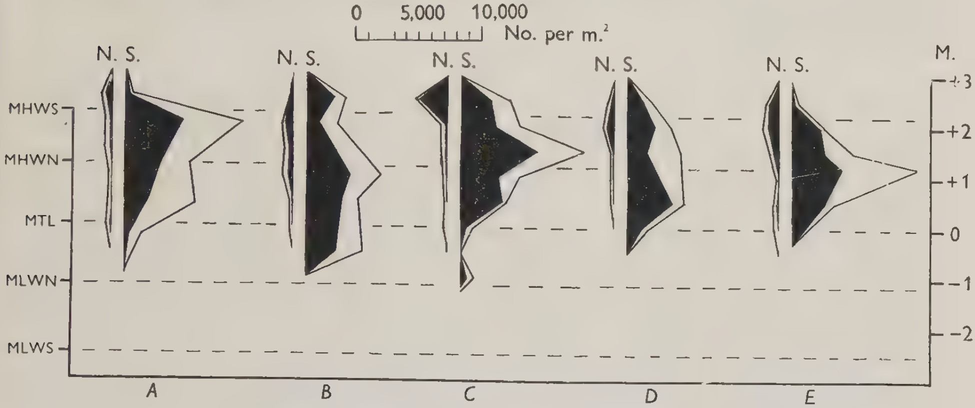

Fig. 7. The distribution of *Littorina neritoides* (■) and *L. saxatilis* (□) on the breakwater.

While *Littorina neritoides* was the dominant littorinid on both sides of the breakwater, the numbers present on the south side were at least three times as great as those on the north side; the maxima found were 1000/m.² at E.H.W.S. on the north side of traverse *C*, and 3000/m.² at M.H.W.N. on the south side of traverse *C*.

L. saxatilis was commonest on the south side of the breakwater, where it constituted half of the littorinid population between M.H.W.S. and M.H.W.N., and reached a density of 3000/m.² on traverse *E*. On the north side the numbers did not reach 500/m.², but the lower limit was some 0·5 m. lower than that of *L. neritoides* (Fig. 7).

It is interesting that *L. neritoides* should be the commoner littorinid on both sides of the breakwater since it is usually found in exposed places only (cf. Evans, 1947*b*). It is possible that there is some factor inimical to *L. saxatilis* on the north side.

Discussion and Conclusions

With the exception of *Lichina*, found only on the south face, and *Osilinus*, found only on the north face, all the species investigated were present on both sides of the breakwater. The factors influencing the distribution of *Lichina* are somewhat obscure, but Evans (1947*b*) has shown some correlation with wave-action; the breakwater evidence, such as it is, would appear to confirm this. *Osilinus* and the other top-shells were scarce even on the north side of the breakwater. They have little power of attachment, and appear very intolerant of wave-action on steep faces or smooth slopes, where they are in great danger of being swept away. They reach their greatest abundance on flat, broken shores, where they may exist under moderately wave-beaten conditions.

The remaining differences between the two sides of the breakwater were restricted to changes in the extent of the zones, or in the abundance of individual species.

Zonation

Except for *Fucus vesiculosus* and *Himanthalia lorea*, most of the plants showed a strong tendency to a narrowing of the zone on the southern exposed side of the breakwater. The more pronounced nature of this narrowing at the western end clearly demonstrates its relation to increasing exposure to wave-action. Perhaps the greatest difference between the two sides of the breakwater was shown by *Fucus spiralis*. However, it is possible that the much lower limit reached by this species on the north side reflects the absence of *Ascophyllum* (the breakwater is apparently too steep for this species) which usually competes with *Fucus spiralis* in sheltered places.

In contrast to the plants, all the animals with the exception of the top-shells occupied a wider zone on the south side than on the north side. At the same time the zone was wider at the west end of the south face than at the east end. Obviously, increasing wave-action causes an extension of the upper and lower limits of these species, but the relationship does not appear to be a linear one. Thus, the wave-action on the south face has up to 10 times the amplitude of that on the north face, while along the south side, the amplitude is some 10–40% greater at the western end. Yet the zones may undergo a greater extension between the east and west ends of the south side than between the north and south sides of any traverse. For example, the zone of *Chthamalus* increases by 20% between the north and south sides of the breakwater, but increases by the same percentage between the east and west ends of the south side. Similarly, with the littorinids and *Patella vulgata*, the increase between the east and west ends of the south side is at least equal to, if not greater than, that between the north and south sides. While it is possible that other factors are involved in the case of *Patella*, we have observed elsewhere that the increase in the wave-action may be out of all proportion to the resulting change in levels. For example, in the Plymouth area, the normal upper limit of *Chthamalus*, excluding the upper parts of the estuaries, is approximately M.H.W.S., yet even in the most exposed places, where wave-action may have increased more than tenfold, it is rare for the species to extend more than a metre above this level.

A more direct relationship to increasing wave-action is shown by the upper limit of *Patella aspera* (Southward, 1953) and possibly of *P. intermedia*, but this could not be demonstrated on the breakwater since too few specimens were found on the north side.

Abundance

The effect of wave-action on algae is comparatively well known (Stephenson, 1939; Evans, 1947*b*), and the breakwater evidence confirms previous investigations. Not only was the plant cover higher on the north side, but the maximum fucoid population occurred at its eastern end, the area with the least exposure to wave-action. In contrast, the lowest cover of all the species of plants investigated was found at the western end of the south side, the area of maximum wave-action.

The relationship of the numbers of the animals to wave-action is not quite so clear. The barnacles were most numerous towards the western end of the south face, but with *Balanus perforatus* and *B. crenatus*, which occur also in sheltered situations, this was probably an effect of the sparser plant cover. The numbers of *Chthamalus*, however, were almost directly related to the extent of the wave-action, and even the few specimens of *Balanus balanoides* present on the breakwater were commonest in the region of maximum wave-action. The present work offers no reason why the barnacles should increase in number with increasing wave-action, but from previous work it seems to be due to a greater initial settlement of larvae (Hatton & Fischer-Piette, 1932). It has been suggested that the increased water movement brings more food to the animals (Moore, 1935) and this may help to maintain the initial density (Southward, 1953).

The abundance of *Patella vulgata* is affected by the nature and amount of the plant cover (Fischer-Piette, 1948; Jones, 1948; Southward, 1953), which can provide both shelter and food, and large populations may exist both on comparatively bare but wave-beaten places and in comparatively sheltered but weed-grown places. In both habitats there appears to be a higher initial settlement of spat (Hatton, 1938; Southward, 1953), but the rate of growth is apparently greater in the algal-covered areas, hence the higher proportion of larger sizes in such places (Fischer-Piette, 1941, 1948).

The numbers of many intertidal organisms are affected by the nature of the substratum. Thus, on the breakwater, *Chthamalus stellatus*, *Patella vulgata* and *P. intermedia* all showed greater numbers on the rough granite than on the smoother limestone. It is possible that this is partly an effect on the settlement, since barnacles are well known to settle more densely on rough surfaces. It is also possible, with the limpets, that the rougher surface (of both the granite and the large barnacle population thereon) may restrict locomotion (Jones, 1948), thus reducing the feeding radius, and permitting the maintenance of a large population.

In conclusion, we can, on the evidence from the breakwater, divide up the organisms dealt with into three groups according to their relations to wave-action. First, there are those species to which wave-action is unfavourable, and which show it by a narrowing of their zone (either a fall in the upper limit, or a rise in the lower limit, or both) and a reduction in their numbers. These are, in approximate order of decreasing sensitivity: *Osilinus lineatus*, *Gibbula umbilicalis*, *Pelvetia canaliculata*, *Fucus spiralis*, *Laminaria saccharina*, *Fucus serratus*, *Laminaria digitata* and *Fucus vesiculosus*. Then there are those species to which wave-action appears favourable, since under its influence their zone is widened and their numbers increased: these are: *Porphyra umbilicalis*, *Lichina pygmaea*, *Chthamalus stellatus*, *Patella intermedia*, *P. aspera*, *Littorina neritoides* and *L. saxatilis*. Finally, there are some species for which the breakwater evidence is inconclusive: *Himanthalia lorea*, *Rhodymenia palmata*, *Balanus perforatus*, *B. crenatus* and *Patella vulgata*.

SUMMARY

This paper describes an investigation into the effects of wave-action on the distribution, zonation and numbers of the commoner intertidal plants and animals living on the Plymouth breakwater.

Comparison of observations of wave-action on the breakwater with local wind records produces an exposure index for the year of approximately 35% which, although similar to a previously calculated index, is much below similarly derived indices for places in the Isle of Man. It would appear that swells and refracted waves are more important at Plymouth.

The wave-action observations showed great differences between the two sides of the breakwater, the height reached by the wash on the south side being more than 10 times as great as that on the north side. At the same time, the wash on the south side was found to be some 10–40% greater at the western end than at the eastern end.

The distribution and abundance of the commoner plants and animals is described and discussed in relation to tide level and wave-action.

Most of the plants, with the exception of *Lichina*, showed their greatest abundance and widest zone on the north face, particularly at its eastern end, which is probably the most sheltered point on the breakwater. *Himanthalia lorea* and *Rhodymenia palmata*, however, showed little differences between the two sides of the breakwater.

The majority of the animals, most notably *Chthamalus stellatus* and *Patella intermedia*, were favoured by wave-action, the numbers being greater and the zone wider on the south side, and especially at its western end, the most wave-beaten part of the whole breakwater. The top-shells, however, were commonest on the north side. The evidence for *Patella vulgata* was inconclusive, but tended to confirm previous evidence that this species is abundant in both weed-grown and wave-beaten places, but that the zone is wider and the shell smaller in the latter situations.

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J. Mar. biol. Ass. U.K. (1954) 33, 21–25
Printed in Great Britain

NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

I. THE PRESENCE OF *DIDEMNUM* (*LEPTOCLINIDES*) *FAERÖENSE* (BJERKAN) IN THE PLYMOUTH AREA

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(Text-figs. 1, 2)

Leptoclinides faeröensis Bjerkan (1905) is a little-known boreal species which has been found only in the Atlantic Ocean (Fig. 1). The most northerly record is from a little south of Spitzbergen, the most southerly from 37° 08′ N., off the North American coast. It occurs on the coast of Norway and in the Faeroe Islands, but it has not been reported nearer to Plymouth than these two localities. Most records are from deepish waters, though it occurs in the sublittoral zone along the Norwegian coast. It was in this zone, at Looe Island (50° 20′ 24″ N., 4° 26′ 53″ W.) near Plymouth, that we found a specimen of this species growing on a rock about 80 cm. below O.D.—just sufficiently low for it to remain covered by a few centimetres of water at the lowest tide of the year, the equinoctial spring tide.

The colony was 13 × 8 mm. across and 3 mm. thick. It was attached by the whole of its lower surface. The position of each zooid was marked by an area of sparse spicules showing darker against the white opacity of the spicule-packed common test. The spicules were concentrated in the upper layers of the colony. The deeper test was yellowish and of a firm consistency, almost free of spicules. The spicules were remarkably even in size, much more so than in most colonies of didemnids; they were 40–50 μ in diameter with a few long conical points; the formula was usually 1, 6, 9 or 3, 6, 9. They are illustrated by van Name (1945, p. 96, fig. 43). There was a single common cloacal cavity which was at the level of the abdomina of the zooids. The thoraces were completely embedded in the test while the abdomina had each a thin sheath of test-substance. Correspondingly, the atrial siphon of each zooid was prolonged backwards into a long funnel to reach the common cloacal cavity. This is the diagnostic generic character. The zooids were arranged vertically to the surface of the colony and each had six lobes to the branchial siphon. There were thirty-two oral tentacles of four orders of size arranged 1, 4, 3, 4, 2, 4, 3, 4, 1. The zooids varied from 1·1 to 2·1 mm. in length. The smallest had only three rows of stigmata, the majority four rows and the few largest had an incipient fifth row as shown in Fig. 2. The number of stigmata in each half-row was 9–12; in the fifth row, where present, this number was only 3–5. There were usually three dorsal languets; a fourth was occasionally present if there was a fifth row of stigmata, though not always even then. The lateral thoracic organ was small, a little less than the height of a single row of stigmata, and lay between the third and fourth rows.

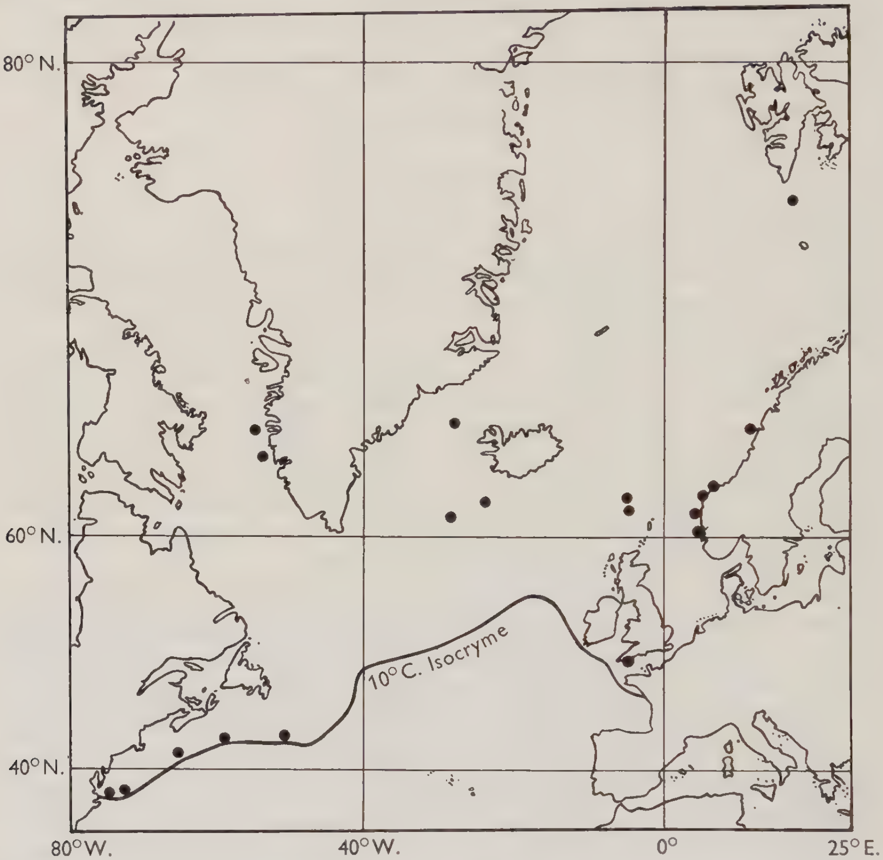

Fig. 1. The recorded distribution of *D.* (*Leptoclinides*) *faeröense*, showing agreement between the most southerly records of the species and the mid-winter sea temperature of 10° C.

The abdomen was little, if at all, larger than the thorax. The neck was sharply constricted. The oesophagus was straight and opened into a globular or squarish stomach. The post-stomach was horizontal and formed the bottom of the gut loop. The mid-intestine was short and sharply delimited from the post-stomach and from the rectum by grooves. The rectum was curved into an abrupt S; the final bend lay to the left of the stomach. The ovary lay entirely within the intestinal loop, the testis to the left side of the loop extending rather behind the mid-intestine. It was undivided. The sperm duct made four or five turns around it before running to the atrium. The atrial siphon was very long and possessed a strong sphincter muscle.

The colony was collected on 18 March 1953, and was not then breeding. The larva is unknown.

The species does not appear to have any synonyms, other than misspellings of the trivial name, and is clearly distinguishable from any other species. For references to the literature see van Name (1945).

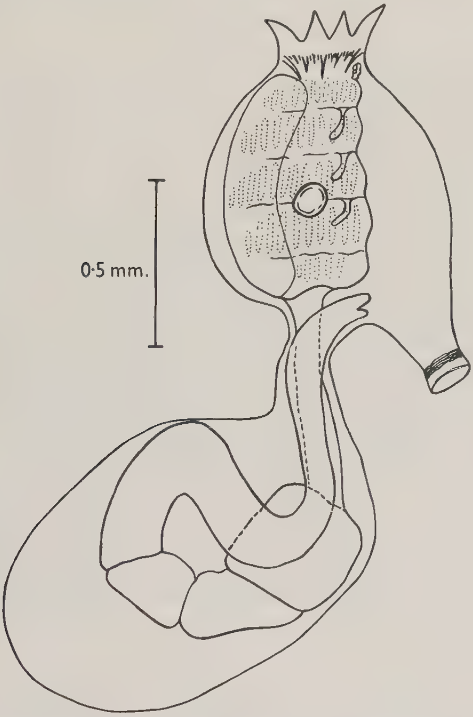

Fig. 2. Drawing from the left side of a large zooid of *D. faeröense*, omitting the reproductive system. Note especially the incipient fifth row of stigmata only present in the largest zooids.

Six species of the genus *Leptoclinides* are known, the north Atlantic *L. faeröensis*, the south Atlantic *L. brasiliensis*, known only from Michaelsen's (1923) description of his type specimen, *L. glauerti* Michaelsen (1930), likewise known only from the type specimen, *L. dubius* (Sluiter), *L. ocellatus* (Sluiter), and *L. madara* Tokioka. The last three of these species are not significantly different, except in the condition of the atrial system, from the subgenus *Polysyncraton* of *Didemnum*. Sluiter's species were indeed described under this genus, but they were separated from it by Michaelsen (1930) and Tokioka (1953). *Leptoclinides madara*, according to its author, may be merely a colour variant of *L. ocellatus*. *L. brasiliensis* is rather similar to *L. faeröensis*, but has an unlobed branchial siphon, and the common cloacal cavity is completely below the level of the zooids so that the atrial siphon is even more prolonged. Michaelsen suggests that the common cloacal cavity may open on the lower side of the colony, but this cannot happen in *L. faeröensis* because the whole lower surface is attached to the substratum. In *L. glauerti* the atrial siphons open direct on to the lower surface of the colony and the common cloacal cavity system is abolished altogether, or represented at most by small local depressions on the lower side into which individual atrial siphons open.

If, then, the more posterior level of the common cloacal system is worthy of generic distinction, how much more should be the complete abolition of the system. If, in other words, the genus *Leptoclinides* has any validity as separate from *Didemnum*, then *Leptoclinides glauerti* should be in a further separate genus. But how valid is the genus *Leptoclinides*? *L. faeröensis*, the type of the genus, differs from *Didemnum* only in the position of the common cloacal cavity and in the associated prolongation of the atrial siphon. The same condition is seen to a lesser extent in *Trididemnum tenerum* and *T. niveum* (see Carlisle, 1953), yet there is no suggestion that the condition is worthy of generic distinction there. Within both *Didemnum* and *Trididemnum* there is great specific and individual variation in the degree of development, the extent and the level of the common cloacal system, and it is for this reason that the genus *Leptoclinum* has been abandoned—the character on which it is based is too variable. Now it is on precisely this character that the genus *Leptoclinides* is based. The distinction does not hold so the genus collapses. *L. faeröensis* and *L. brasiliensis* should be received into the genus *Didemnum*, retaining subgeneric rank, while *L. glauerti* requires a new genus for its reception. The name *Sinecloaca* seems appropriate as expressing the lack of common cloacal cavity in this form.

Sinecloaca n.gen.: an ascidian genus presenting typical didemnid characters possessing usually four rows of stigmata; sperm duct coiled around testis; differing from *Didemnum* Savigny in the reduction, apparently secondary, of the common cloacal cavity system to a few local depressions on the lower surface of the colony into which individual atrial siphons open direct to the sea, and in the corresponding elongation of the atrial siphons to open in this manner on the side of the colony opposite to the branchial siphons.

Type species: *Sinecloaca glauerti* (Michaelsen) (= *Leptoclinides glauerti* Michaelsen, 1930).

The remaining species of the abandoned genus *Leptoclinides* Bjerkan become *Didemnum* (*Leptoclinides*) *faeröense* (Bjerkan) and *D.* (*Leptoclinides*) *brasiliense* (Michaelsen), while *L. dubius*, *L. ocellatus*, and *L. madara* go back to *Polysyncraton* where they belong—*Didemnum* (*Polysyncraton*) *dubius* (Sluiter), *D.* (*P.*) *ocellatus* (Sluiter), and *D.* (*P.*) *madara* (Tokioka) or *D.* (*P.*) *ocellatus* (Sluiter) forma *madara* (Tokioka).

SUMMARY

Didemnum (*Leptoclinides*) *faeröense* Bjerkan is present in Plymouth waters. The adult is briefly described. The genus *Leptoclinides* Bjerkan is not valid and two of its six species should be included in *Didemnum* as a separate subgenus, a third species, *L. glauerti*, must remain separate in a genus of its own for which the name *Sinecloaca* n.gen. is proposed, while the remaining three species are returned to the subgenus *Polysyncraton*.

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J. Mar. biol. Ass. U.K. (1954) 33, 27–32
Printed in Great Britain

NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

II. THE NUMBER OF ROWS OF STIGMATA IN *DIDEMNUM GELATINOSUM* MILNE EDWARDS AND IN *DIDEMNUM MACULOSUM* (MILNE EDWARDS)

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Milne Edwards (1841) established the genus *Leptoclinum* for those Didemnidae which possessed a largely expanded common cloacal system. His type of the genus was *L. maculosum* which is now placed in the genus *Didemnum* Savigny (1816). Other species were *L. asperum* and *L. durum* which are now regarded as mere forms of *Didemnum maculosum* (e.g. Harant & Vernières, 1933); *Leptoclinum fulgens* which is closely related (see Harant & Vernières), if not a mere colour form of *Didemnum maculosum*; *Leptoclinum listerianum* which has been transferred to the genus *Diplosoma* Macdonald; and *Leptoclinum gelatinosum*. This last has often been considered synonymous with *Diplosoma listerianum*, but my reasons for disagreeing with this assumption are elsewhere expressed (Carlisle, 1953). Milne Edwards did not of course describe the course of the sperm duct by which *Diplosoma* is distinguished from *Didemnum*. In the absence of a description of this diagnostic character we are reduced to a consideration of numerous details of the anatomy of *Leptoclinum gelatinosum* in order to decide where to place it in the classificatory scheme. When such a point-by-point comparison of shape and curvature of the gut, position of anus, form of atrial and buccal apertures, nature of test, etc., is made, it appears that the one point in which the drawings and descriptions given by Milne Edwards differ between *L. gelatinosum* and *Didemnum gelatinosum* Milne Edwards (1841) is the presence of a common cloacal system in the former and its absence in the latter. But the presence or absence of a common cloacal system is no longer regarded as a generic distinction (see, for example, van Name, 1945). Berrill (1950), on the basis of one specimen, and presumably with Milne Edwards's authority, states that *D. gelatinosum* has no common cloacal system. I am unable to agree with this statement. Large colonies of *D. gelatinosum* collected and identified by myself, and other large colonies collected and determined by Miss P. Kott (and in the type collection of this laboratory), frequently contain a large common cloacal system stuffed with faecal pellets, whereas small colonies are without such cavities. It seems to me then, that we have no clear grounds for separating *Leptoclinum gelatinosum* Milne Edwards from *Didemnum gelatinosum* Milne Edwards (1841) and, so far as it is possible to recognize the species from his descriptions, these are most probably synonymous, particularly as his drawings and descriptions of *D. gelatinosum* are based on a small specimen with only a small number of zooids, while his description and drawings of *Leptoclinum gelatinosum* are evidently based on a much larger colony. The identification of the one with the other can of course never be certain. Certainly, however, the description of *L. gelatinosum* is unlike that of any other species of didemnid found in the English Channel.

One thing stands out as strange in Milne Edwards's drawings and descriptions—he states that in *L. gelatinosum*, 'Le sac branchial est garni de cinq rangées de fentes stigmatiformes' (1841, p. 300), and he illustrates it so. Similarly, he states of *Didemnum gelatinosum*, 'Le thorax est gros et n'offre que cinq rangées transversales de stigmates branchiaux' (p. 296). He figures it with sometimes three, sometimes four, and sometimes five rows of stigmata (his plate 7, figs. 5*a–e*), so that his 'n'offre que cinq rangées' must mean 'has no more than five rows,' rather than 'has only five rows'. How is all this to be squared with the usual statement that the Didemnidae possess either three or four rows of stigmata—e.g. van Name's statement (1945, p. 78) 'There are (apparently always) either three or four rows of stigmata'—or with the diagnostic difference usually offered between *Didemnum* and *Trididemnum* that the former has four rows and the latter only three rows of stigmata? Clearly, since the species *Didemnum gelatinosum* is ascribed to Milne Edwards this matter requires a fresh investigation. If the species that we now call *D. gelatinosum* Milne Edwards always possesses four rows of stigmata then it cannot truly go by this name in view of Milne Edwards's deliberate statements. At the same time it would be as well to examine other species ascribed to Milne Edwards to determine the situation there.

Two species of *Didemnum* are to be found in the Plymouth area in abundance, *D. gelatinosum* and *D. maculosum* auctt. (?Milne Edwards). An examination of a large number of colonies of these two species has shown that the majority of zooids possess four rows of stigmata apiece. Before the breeding season commences, however, when the colonies are growing actively by vegetative means, the zooids around the edges of the colonies are much smaller. These zooids have only three rows of stigmata each, and usually one or two less than usual in each row. During the period of sexual reproduction the number of these smaller zooids is progressively less, and successive tracing of the outline of colonies at Salcombe (Devon) at monthly intervals has shown that during this season growth in area of the colonies is almost at a standstill. At any season of the year in colonies with more than about forty zooids one can usually find one or a few thoraces with five rows of stigmata in both species. Sometimes, when a colony, from the abundance of the faecal pellets and from the general appearance, seems particularly well fed, quite a high proportion of the zooids possess five rows of stigmata, even as high a proportion as 15%

when the animals are not in the breeding season. Towards the end of the breeding season, when the larvae are fully developed, the thoraces of the zooids are resorbed and only abdomina, larvae, and faecal pellets are to be seen in the colonies.

The zooids around the edges of the colony are always smaller than those nearer the centre. The difference of size is more pronounced in the thorax (which, in a zooid from the centre of the colony, may be 2½ times as large [linear measure] as in one from the periphery) than in the abdomen (which is rarely more than 1½ times as big). The difference in size of the abdomina is reflected in that of all the organs. In particular, the spermduct makes fewer turns around the testis in the smaller zooids, and the mature ovum is smaller. Correspondingly, the larva developed from a smaller ovum, derived from a smaller peripheral zooid, is smaller than one produced from a larger central zooid, and such a smaller larva is furnished with only three rows of stigmata instead of the usual number of four which is found in the larvae in the centre of the colony. Larvae from the centre of the colony of *D. gelatinosum* have five pairs of anterior ectodermal ampullae, while the smaller peripheral ones have only four pairs; in *D. maculosum*, which has a smaller larva, the central larvae have four pairs of ampullae, or rarely five, while those from the periphery have only three (see also Kott, 1952). Further differences are to be seen in the anatomy of the zooids from the centre and from the periphery of the colony. The course of the gut is less circuitous in the smaller peripheral zooids. Thus in *D. maculosum* the rectum in the larger central zooids makes two sharp bends to form a shallow S (see Milne Edwards, 1841; Millar, 1949); in the smaller zooids from the periphery the course of the rectum is almost straight. This may perhaps be correlated with the larger amount of food that the larger thoraces may supply to the central zooids, which requires a longer gut for its digestion. The mid-intestine is relatively rather larger in the smaller zooids, though absolutely slightly smaller.

Evidently Milne Edwards was correct in his statement that *D. gelatinosum*, 'n'offre que cinq rangées transversales de stigmates branchiaux' (1841, p. 296), and that in *Leptoclinum maculosum* the structure of the zooids 'est essentiellement la même' (p. 298). His descriptions and figures suffer simply from the general fault of those of his period—he always worked from the largest and best developed, never from the typical or average specimen. This is true not merely of his descriptions and figures of didemnids but also of other ascidian species. More recently the emphasis has been on examining average specimens; in determining the species of a colony of a didemnid it is usual practice to cut off and dissect a 'typical portion', neither too thick nor too thin, nor too near the edge. Such a portion would contain almost entirely zooids with the usual number of rows of stigmata for the colony—four in *Didemnum*. It is notoriously difficult to count the number of rows of stigmata, especially in dead and preserved specimens, and indeed neither Michaelsen (1923) nor Hartmeyer (1924) was able to determine the number of rows in the species which the former named *D. helgolandicum*. Accordingly, it is usual to examine a number of zooids and discount as impossible to observe properly any which seem to show an unusual number of rows of gill-slits. Thus any zooids in a *Didemnum* colony with other than four rows of gill-slits would be unlikely to be taken for examination and if examined would most probably be discounted. It is only when one examines living zooids of *Didemnum* species that the number of rows of gill-slits is seen, with practice, clearly enough for one to be unable to mislead oneself with a belief that unusual zooids are actually ones which are difficult to observe correctly and not really unusual at all. It comes as rather a shock to see five rows of gill-slits in a living zooid of *D. maculosum*, with each slit clearly outlined by a ring of beating cilia, and to realize that preserved ones, previously discarded as poorly preserved and misleading, really did show five rows after all. It may be noted that Milne Edwards (1841, p. 219), unlike most of his successors, examined his specimens alive.

The account given by Salfi (1933, 1950) of budding in the Didemnidae gives some explanation of the way in which the number of rows of stigmata may increase with the age of the zooid. He describes budding in these forms as proceeding by the formation of half-buds. The oesophageal region of a zooid buds off separate thorax and abdomen, either together or at different times. Either of these half-buds can complete itself by the formation of the other half, or two halves formed simultaneously and near together may combine. But more thoracic buds are formed than abdominal buds and not all of these go to form fresh zooids. Some of them are used by the parent zooid to replace its own thorax which is worn out. The old thorax is resorbed and the newly budded one takes its place, or rather the other way round, for the new one begins to function before the old thorax is resorbed. This may apparently happen several times in the life of a single zooid. This I can confirm from my own observations. At a time when the colony of *D. maculosum* is growing most actively by vegetative means, most new zooids are formed from abdominal buds which then form their own thoraces. Such buds are small, chiefly around the edge of the colony and most have only three rows of stigmata. As they grow larger a new thoracic bud is formed, by each such zooid, which takes over the function of the old thorax which is now too small and worn out. This new thorax has the typical four rows of stigmata. This may happen several times as the zooid grows larger until when it is very large indeed a new thorax may be large enough to have five rows. The limiting factor for the number of rows of stigmata seems to be the size of the thorax at the time when it begins to take over its functions. Once it is functioning there seems to be no further development of fresh rows of stigmata, although there may occasionally be an increase in the number in one row. When the breeding season starts most of the energy of the colony goes to the production of embryos. New budding and growth of the colony stops and vegetative growth seems confined to necessary repair work: zooids may replace their thoraces but not form entirely new buds. This may account for the decrease in number of zooids with three rows of stigmata during the breeding season. Small zooids naturally produce small eggs and the larger, older zooids produce larger eggs. Again the size of the thorax of the larva seems to determine how many rows of stigmata it shall have—as many rows develop as there is room for. Small larvae have only three rows, larger ones four. It is probable that in the past larvae with only three rows of stigmata from *D. maculosum* have been interpreted as not fully developed, but this is not so, for free-swimming, small larvae may possess only three rows up to metamorphosis, beyond which I have not observed them further. Such differences in the structure of larger and smaller larvae may be compared with similar differences which I have observed in such oviparous species as *Phallusia mammillata* (Cuvier), between larvae obtained by artificial fertilization of eggs from the oviduct and of eggs from the ovary, which are always slightly smaller and produce larvae with a simplified sensory system and other simplifications and reductions in their anatomy.

The situation with respect to the number of gill-slits is similar in *Didemnum* (*Leptoclinides*) *faeröense* (Bjerkan) as described in part I of these notes (Carlisle & Carlisle, 1954). In *Diplosoma* spp., however, I have never seen other than four rows of stigmata in any zooid.

Summary

The number of rows of stigmata in three species of *Didemnum* varies from zooid to zooid within the colony. Peripheral, newly formed, small zooids have only three rows, most zooids have four rows, while a few very large ones have five rows. This is probably a result of the replacement of the thorax by partial budding during the life of the zooid, accompanied by a steady increase in size. The number of rows of stigmata seems to be always four in *Diplosoma* species. The importance of examining living specimens is stressed.

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J. Mar. biol. Ass. U.K. (1954) 33, 33–48
Printed in Great Britain

THE ANNUAL GROWTH AND REPRODUCTIVE CYCLE OF THE ASCIDIAN *DENDRODOA GROSSULARIA* (VAN BENEDEN)

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(Text-figs. 1–11)

This paper is the second of a series dealing with the annual cycle of growth and reproduction in British ascidians; a previous paper (Millar, 1952) dealt with the species *Diplosoma listerianum* (Milne Edwards), *Ciona intestinalis* (Linnaeus), *Ascidiella aspersa* (Müller), and *Botryllus schlosseri* (Pallas).

The present investigation is concerned with the growth, the course of sexual reproduction and establishment of new generations, and the histological changes in the gonad throughout the year, in the ascidian *Dendrodoa grossularia* (family Styelidae).

Samples were examined from two widely separated localities: Fambridge in the River Crouch, Essex; and Farland Point, Isle of Cumbrae, in the Firth of Clyde. The specimens from Essex were dredged from a depth of about 2 m. (below L.W.O.S.T.), and were attached to the empty shells of the European oyster, *Ostrea edulis* L. and to the shells of *Crepidula fornicata* L. The specimens from the Firth of Clyde were attached to stones on sheltered parts of the shore. The animals here extended over a zone of the shore from about Chart Datum + 2·5 ft. (0·76 m.) to about Chart Datum + 7·5 ft. (2·29 m.). Only in very sheltered positions, such as under large boulders, did *Dendrodoa* occur in abundance. The two habitats were thus very different in nature, one being sublittoral on the south-east coast of Britain and the other being littoral on the north-west coast. The value of comparisons is limited by the different nature of the conditions to which the sublittoral and the littoral populations were exposed.

The observations on growth in the Clyde extended over most of 1951 and 1952, and nineteen samples were taken. The ten samples in 1952 were used for examination of breeding condition, and of histological state of the gonad. Eleven samples from Essex covered the period December 1951–December 1952, and supplied data on growth, breeding condition, and histological state of the gonad.

I wish to thank Mr M. N. Mistakidis and Mr G. Duncan Waugh, of the Ministry of Agriculture and Fisheries, for collecting and sending the samples from Essex, and for providing temperature records for that area.

GROWTH

About 200 animals in each sample were measured, to the nearest 0·1 mm., the antero-posterior length of the body being taken as a measure of the size. Care was taken to measure the length of the body itself, excluding the basal expansion of test which is very variable in extent. The length of the body is not an entirely satisfactory measure of the size since the ratio of length to height varies to some extent with the habitat, but length was found to be the best practical measure available.

The Essex population

Histograms of the distribution by size of *D. grossularia* in the samples from Essex during 1952 are shown in Fig. 1. The samples of 15 December 1951 and 15 January 1952 represent the winter state of the population. There are two clearly separable components: (i) individuals up to about 1·5 mm. long, and (ii) the remaining individuals from about 1·5 mm. to about 8·5 mm. in length.

There is no clear indication of spring growth until after 25 March 1952, although it may have started somewhat earlier than this. Considerable growth occurred in the animals of the 0–1·5 mm. group, between late March and early May, and continued throughout June and July so that they had attained a length of 4·0–5·5 mm. by 22 July 1952. No significant change is seen in the form of the histograms, for sizes over 4·0 mm., in the period up to 22 July 1952. This is due to the growth of small animals up into the size groups over 4·0 mm. and, we may suppose, little growth in the large animals together with death of the oldest ones.

During May and to a less extent in April and June the spring settlement of 1952 took place. This period of settlement is confirmed by the presence of larvae in breeding individuals (see pp. 38–40). Little or no settlement took place in August.

The autumn settlement occurred mainly in September, October and November, and these animals attained a length of up to 1·5 mm. before growth stopped for the winter. The histogram of 5 December 1952 again shows the winter structure of the population, with two components: (i) individuals under 1·5 mm. representing the autumn 1952 settlement, and (ii) individuals from about 1·5 mm. up to the maximum size, representing the spring 1952 settlement together with an unknown proportion of the 1951 settlements.

During a considerable part of the year, from the time of establishment of the autumn generation through the winter and on into the spring there are present at least three groups of individuals of separate origin. Thus in the autumn of the year n these groups represent (i) the autumn settlement of the year n, (ii) the spring settlement of the year n, and (iii) the autumn settlement of the year $n-1$, together with some unknown proportion of the settlements of spring $n-1$, and of the year $n-2$.

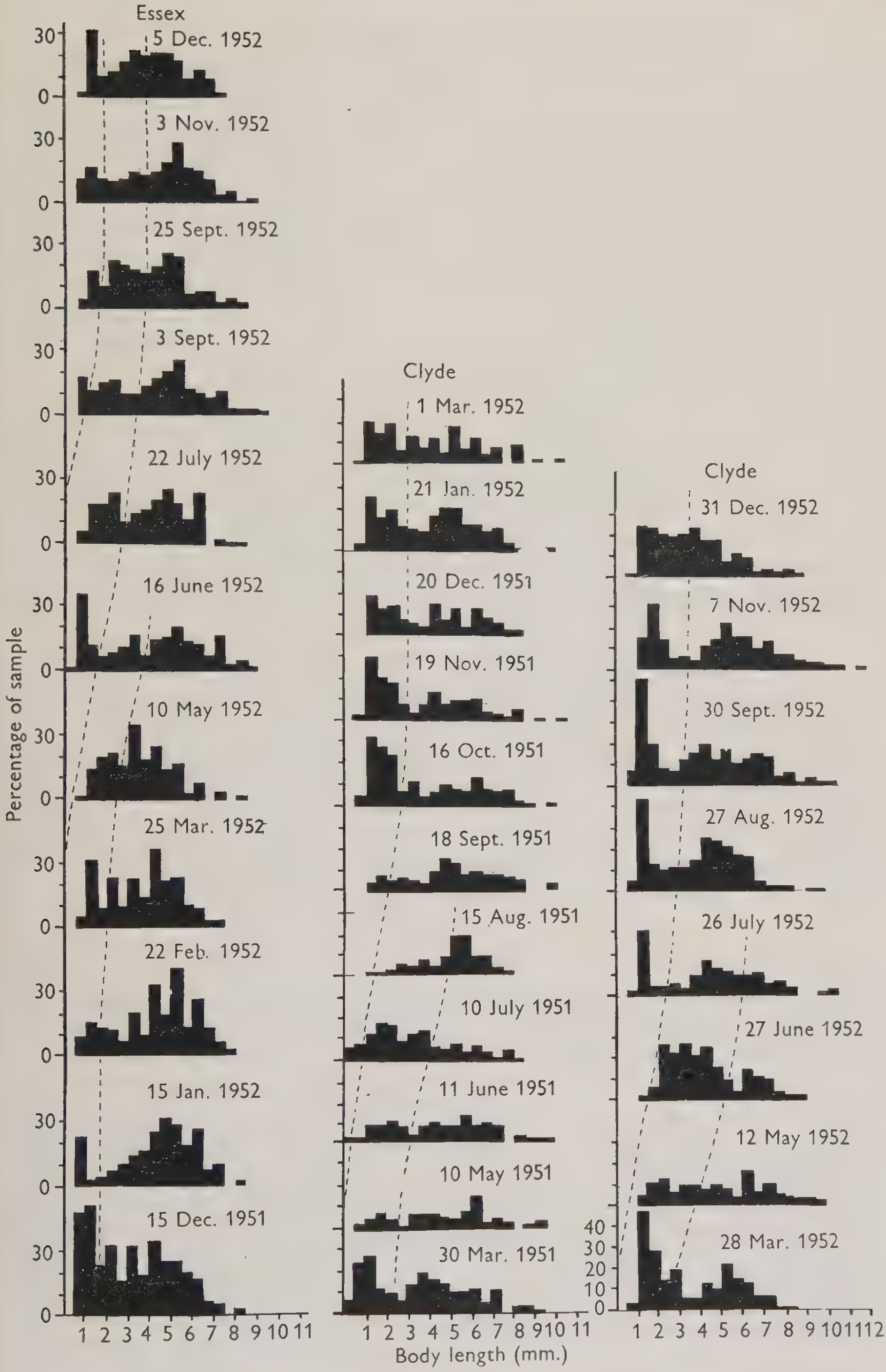

Fig. 1. Size-frequency histograms of *D. grossularia* samples from Essex and the Clyde.

The Clyde population

Histograms of the distribution by size of animals in the samples from Farland Point, Cumbrae, during 1951 and 1952 are shown in Fig. 1. There was a tendency for these animals to attain a greater length than the specimens from Essex, which can be attributed to their flatter shape in the more exposed littoral habitat.

The winter state of the population is seen in the histograms for 20 December 1951 and 7 November 1952. The figure for 7 November 1952 shows two groups of animals: (i) individuals up to about 3·5 mm. long, representing the single settlement which lasted from early summer until late autumn 1952 (see pp. 38–41); and (ii) the remaining, larger, animals representing the settlement of 1951 and any survivors of the settlement of 1950.

The Clyde population resembled that of Essex in the period and extent of growth during the year. There was no observable difference in the time of onset of spring growth, but the technique of measurement and the frequency of sampling may not have been capable of detecting it. The main difference in the Clyde population was its single long period of settlement resulting in a new generation spread over a considerable range of sizes. Each year therefore contributes a single component to the population, instead of two as in Essex.

General Conclusions on Growth and Life Span

The changing structure of the populations is indicated in Fig. 1 by broken lines representing the probable separation of the different components.

In both localities it appears that animals which settled as larvae in any given year attain a length of up to 3·0 or 3·5 mm. by the winter of that year. By the following summer many of them have grown to their maximum size, and during the next spring or summer most of them probably die. They may thus attain an age of 18 months to 2 years.

Reproduction

Asexual reproduction by budding occurs in several groups of ascidians, but in *Dendrodoa* reproduction is entirely sexual.

The Gonad

D. grossularia has a single long hermaphrodite gonad on the right side of the body, attached to the inner surface of the ventral body wall, parallel to the endostyle. The testis lobes occupy the ventral and lateral parts of the gonad, and the ovary occupies the remaining, dorsal part (Fig. 6). Along the dorsal surface of the gonad is a series of male openings, to each of which converge the individual sperm ducts from a number of adjacent testis lobes. The single oviduct opens into the posterior part of the atrial cavity.

Brood Filaments and the Retention of Developing Eggs

When the eggs are extruded from the oviduct they are not carried away by the exhalent stream of water out through the atrial siphon. Instead they are retained within the atrial cavity by a group of specially developed filaments which do not seem to have been described and which may be called the brood filaments. These filaments, which are numerous and closely spaced, stretch across the atrial cavity from the outer surface of the posterior part of the branchial sac to the inner surface of the body wall opposite (Fig. 2). They are probably homologous with the trabeculae which pass from the branchial sac to the body wall in other parts of the body, and which maintain the branchial sac in position. Since the opening of the oviduct lies immediately ventral to this mass of brood filaments, the eggs as they are shed become entangled amongst the filaments. Here they are fertilized and remain during subsequent development to the larval stage. The larvae, however, when fully developed tend to become free of the brood filaments and to spread into the adjacent part of the atrial cavity, on the right. Eggs and larvae are prevented from passing into the left part of the atrial cavity by a vertical mesentery uniting the body wall to the oesophagus, stomach and posterior end of the branchial sac. The liberation of the larvae from the entangling brood filaments may be due both to muscular movements of the larvae and to the pressure of increasing numbers of eggs shed from the oviduct. When thus freed from the

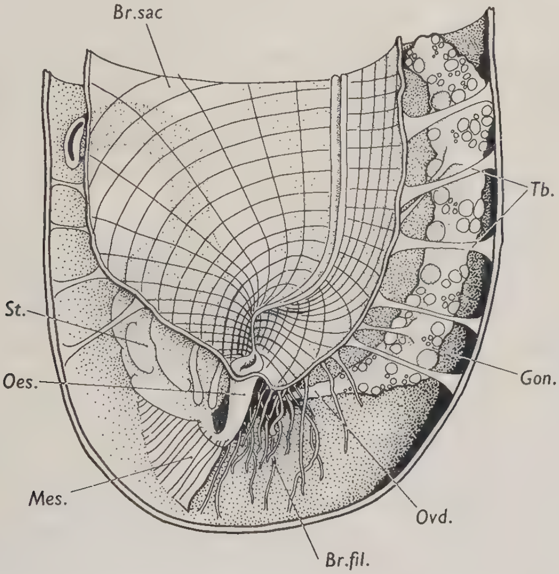

Fig. 2. Ventral half of the posterior part of the body of *D. grossularia*. *Br. sac*, branchial sac; *Br.fil.*, brood filaments; *Gon.*, gonad; *Mes.*, mesentery; *Oes.*, oesophagus; *Ovd.*, oviduct; *St.*, stomach; *Tb.*, trabeculae.

brood filaments the larvae can be carried by the exhalent current to the exterior. The mechanism ensures that it is the most advanced larvae, that is those developed from eggs first shed from the ovary, that are freed from the brood filaments to pass to the exterior, while the younger developing eggs are still retained.

The brood filaments are often found to be attached only at one end, usually to the outer wall of the branchial sac, a condition resulting from their being broken by the pressure of large numbers of eggs.

The Course of Breeding

In each sample during 1952, fifty individuals were dissected, ten in each of the size groups 3–4, 4–5, 5–6, 6–7, and 7+mm. The eggs and larvae in the atrial cavity were counted.

The Essex Population

Fig. 3 shows the percentage of individuals with eggs or larvae in the atrial cavity, in each of the size groups 3–4, 4–5, 5–6, 6–7 and 7+mm., during 1952. Animals under 4·0 mm. in length took practically no part in breeding. The remaining groups all had a rather similar pattern of breeding activity. Eggs were first shed into the atrial cavity during March when, although 30% of the specimens over 7·0 mm. carried eggs, no larvae were yet present. On 10 May 1952 all specimens over 6 mm., and many between 4·0 and 6·0 mm., were breeding. This maximum was followed by a sharp decline, many larvae having escaped to the exterior while few or no further eggs were shed from the ovary. The decline continued and produced a minimum in July, when breeding almost completely stopped. A further liberation of eggs from the ovary starting in August led to a second period of larval development from August to November. This was followed by a decline to the winter non-breeding state of the population.

The Clyde Population

Fig. 4 shows the course of breeding at Farland Point, Cumbrae, during 1952. As in Essex, individuals under 4·0 mm. in size took very little part in breeding, and amongst the Clyde population only a small percentage of the 4–5 mm. group bred. Animals from 5·0 to 7·0 mm. started to breed in April or early May, had one maximum of breeding activity in June, a second in September or October, and then returned to the winter non-breeding state. In the 7+mm. group, however, there was no reduction in the percentage of animals breeding in July and August, such as was observed in smaller animals.

Intensity of Breeding

The mean number of eggs and larvae per breeding individual may be taken as a measure of the intensity of breeding, and Figs. 3 and 4 also show the variation in these numbers during 1952. The rise and fall in breeding intensity coincided with the fluctuations in the percentage of animals breeding, closely in Essex, and approximately in the Clyde.

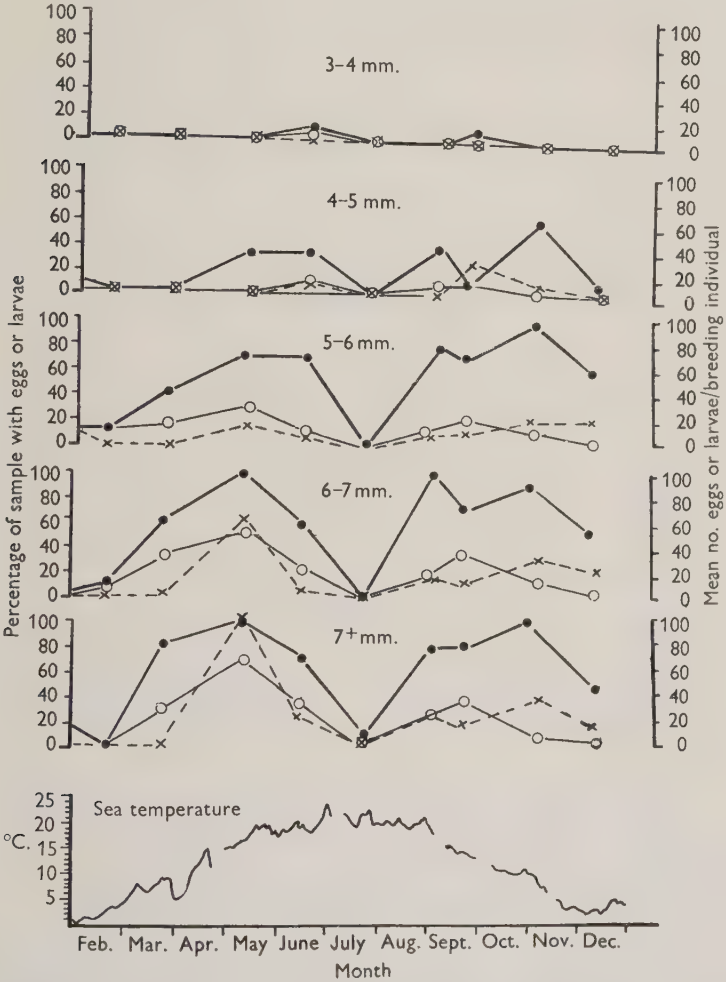

Fig. 3. Breeding of *Dendrodoa* of different sizes in the Essex samples. ●—●, percentage with eggs or larvae; ○—○, mean number of eggs per breeding individual; ×—×, mean number of larvae per breeding individual.

The Essex Population

In all animals over about 5·0 mm. long the intensity of breeding in May exceeded that in autumn. The relation between the length of the body and the number of eggs and larvae found in the atrial cavity is shown in Fig. 5. In general, breeding started at a body length of between 4·0 and 5·0 mm. and increased in intensity with increasing body length. The greatest number of eggs plus larvae found in any individual was 266.

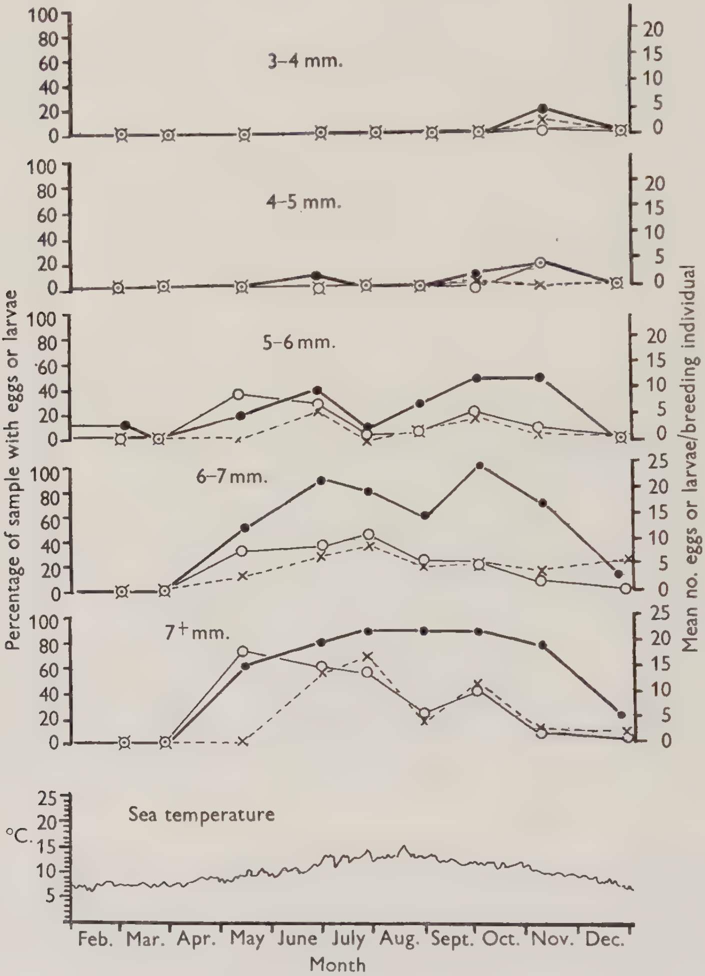

Fig. 4. Breeding of *Dendrodoa* of different sizes in the Clyde samples. ●—●, percentage with eggs or larvae; ○—○, mean number of eggs per breeding individual; ×—×, mean number of larvae per breeding individual.

The Clyde Population

The first maximum in breeding intensity, in July, was greater than the second maximum, in September or October. It has already been noted that there was a slight depression in breeding activity in summer, observed both in the percentage of breeding individuals and in the breeding intensity, in animals of 5·0–7·0 mm. In animals over 7·0 mm. the percentage of breeding individuals remained high throughout the summer, but the intensity of breeding dropped in August, to rise again in September.

Fig. 5 shows the relation between the length of the body and the number of eggs and larvae in the atrial cavity. Very few eggs were produced by animals under 6·0 mm., and in larger animals the number of eggs increased with increasing body length.

The intensity of breeding was much less in the Clyde population than in Essex. The greatest value of the mean number of eggs plus larvae per breeding individual at the height of the reproductive season was only thirty-two in the Clyde, but 172 in Essex, in specimens over 7·0 mm. The greatest number of eggs plus larvae found in any specimen in the Clyde population studied was fifty-five, compared with 266 in Essex. These figures indicate that breeding was about five times more intense in Essex than in the Clyde.

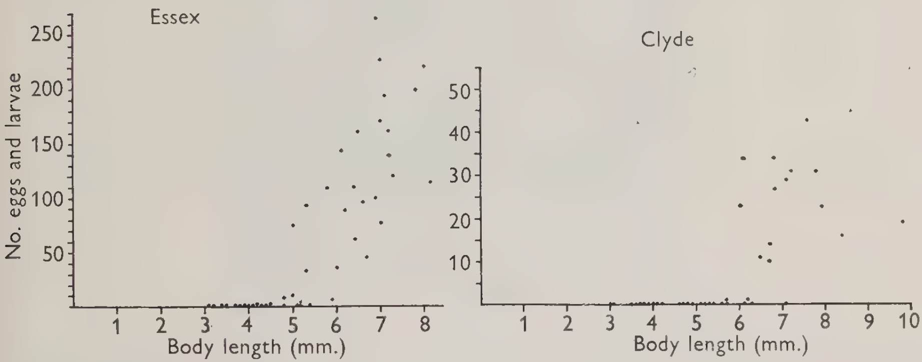

Fig. 5. Relation between length of body and the number of eggs and larvae in the atrial cavity, of specimens from Essex (10 May 1952) and the Clyde (26 July 1952).

Histological Condition of the Gonad throughout the Year

In each of the ten samples taken during 1952 the gonads of ten specimens from each of the size groups 5–6, 6–7 and 7+mm. were fixed in Bouin, sectioned at 12 μ, stained in Ehrlich's haematoxylin and eosin, and examined. Little variation was found in the condition of gonads from the different size groups and the following account therefore applies in general to all mature animals.

The Essex Population

In the overwintering condition the testis lobes consisted of a solid central mass of cells with 'resting' nuclei, and a narrow peripheral layer of phagocytes with dark-staining contents (Fig. 6). There were no spermatozoa or spermatids.

The ovarian part of the gonad had germ cells ranging from a small size up to oocytes 60–120 μ in diameter. None of these oocytes had yet acquired yolk.

There were also present the remains of yolk-laden eggs which had been retained in the ovary at the end of the previous breeding season, and which had been attacked by phagocytes.

The changes which transformed the gonad from this quiescent state to the height of its breeding activity, and the subsequent changes leading again to the overwintering condition, can be best represented graphically (Figs. 7 and 8). During the late winter and early spring the nuclei of the undifferentiated testicular cells started to divide, and by March all specimens had at least some fully developed sperm within the now hollow testis lobes. Meanwhile the peripheral phagocytes had almost completely disappeared from the testis.

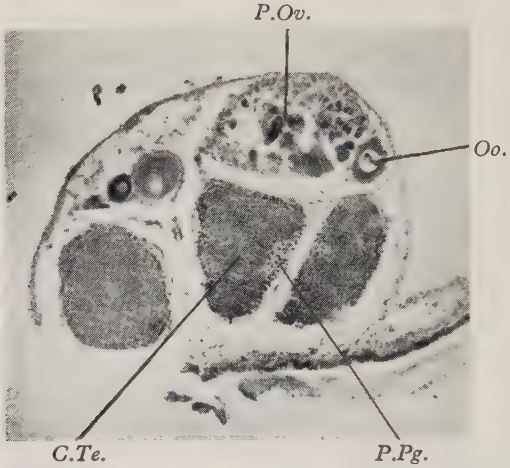

Fig. 6. Photomicrograph of transverse section through gonad in the overwintering condition (Essex, 15 January 1952). 12 μ. Ehrlich's haematoxylin and eosin. *C.Te.*, central part of testis lobe; *Oo.*, oocyte; *P.Ov.*, phagocytosed ovum; *P.Pg.*, peripheral phagocytes of testis.

During the period January–March the ovarian part of the gonad had also undergone changes. The remains of the previous year's unshed eggs disappeared by the completion of the process of phagocytosis which had been slowed down or halted in winter. Oocytes increased in size and yolk started to appear in the protoplasm, so that by the end of March all gonads had yolk-laden oocytes. In early May the gonad reached its fullest development (Fig. 9).

When spawning was intense, sperm began to disappear from the testis lobes, and at the same time phagocytes started to invade the testis. The number of eggs with yolk fell sharply as a result of ovulation, and residual eggs, together with almost ripe oocytes, were attacked by phagocytes (Fig. 9). Resorption of residual germ cells was rapid in July; phagocytes retired to the periphery of the testis lobes and disappeared, and the remains of unshed eggs were also quickly removed.

During August and September the gonads were again brought to breeding condition by a series of changes similar to those that occurred in late winter and early spring. After the autumn spawning the gonads were returned to their overwintering condition by the same processes that followed the earlier spawning.

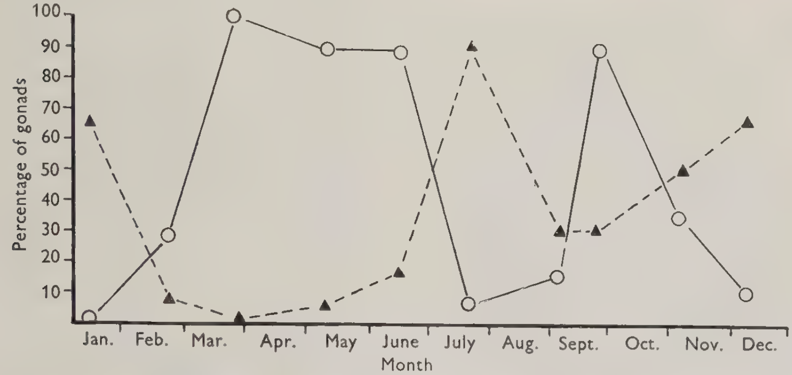

Fig. 7. Histological condition of ovary throughout the year in the Essex samples. ○—○, percentage of gonads with yolk-laden eggs; ▲—▲, percentage showing phagocytosis of eggs.

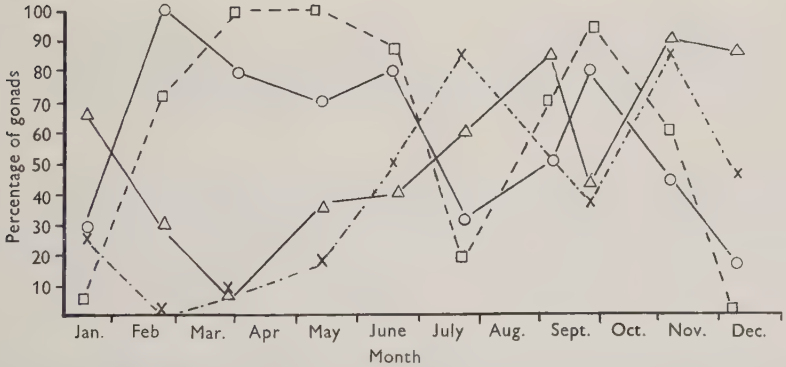

Fig. 8. Histological condition of testis throughout the year in the Essex samples. ○—○, percentage of gonads with dividing male nuclei; □ - - - □, percentage with sperm; △—△, percentage with phagocytes in peripheral part of testis; ×—×, percentage with phagocytes in central part of testis.

The Clyde Population

Figs. 10 and 11 show the cycle of changes in the gonads of the specimens from Farland Point, Cumbrae. The overwintering condition and the condition at the height of the breeding season were the same in the Clyde as in Essex. But in the Clyde there was relatively little suppression of breeding at any time in the summer, and correspondingly little phagocytosis and reversion of the gonad towards a non-breeding state, either in the testicular or ovarian part. The extent of these processes can be seen in the trend of the graphs in August.

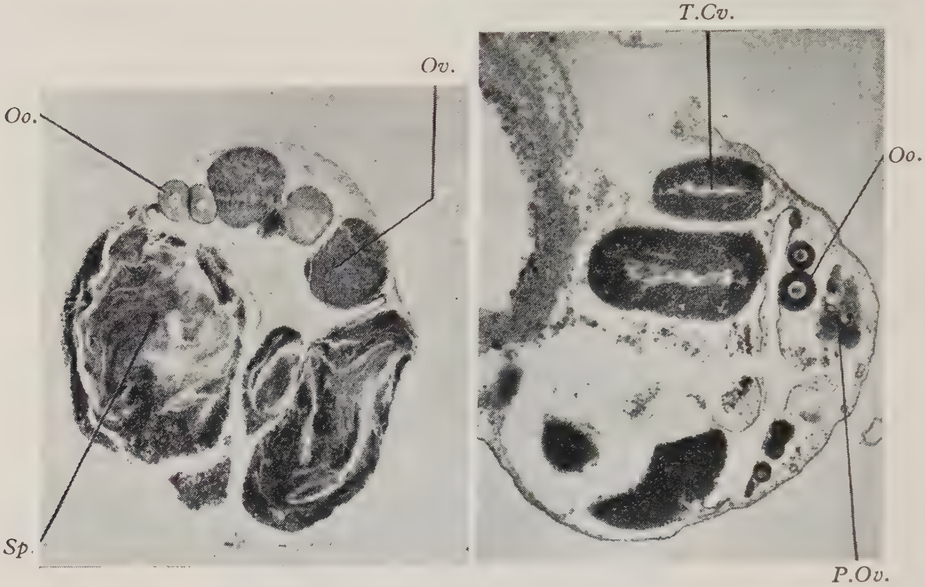

Fig. 9. Left: photomicrograph of transverse section through gonad at fullest development (Essex, 10 May, 1952). Right: after discharge of eggs and sperm (Essex, 16 June 1952). (The dorsal side is to the right in this photograph.) 12 μ. Ehrlich's haematoxylin and eosin. *Oo.*, young oocyte; *Ov.*, ovum with yolk; *P.Ov.*, phagocytosed ovum; *Sp.*, sperm in central part of testis lobe; *T.Cv.*, central cavity of testis lobe.

The Effect of Exposure to Air on the Development of the Gonad

It seemed possible that the difference in breeding behaviour in the two localities might be partly due to the fact that the Essex animals were sublittoral, whereas the Clyde animals were littoral and therefore exposed daily for varying periods. Samples of the Clyde population were therefore taken on 6 March 1953 and 11 May 1953, from a place where *Dendrodoa* extended from low-water mark to a considerable distance up the shore. On each of these dates one sample was taken from the level of Chart Datum +2·2 ft. and one from the level of Chart Datum +7·1 ft. The lower position has a mean exposure of 3·0 hr. per day and the upper position 13·7 hr. per day. The gonads were sectioned and examined as in the main series of samples, and the results are shown in Table I.

The number of gonads examined (about twenty in each of the four samples) was rather small, but the results leave little doubt that a difference in level of 4·9 ft., on the Clyde shore in question, had only a slight effect on the rate of gonad development in spring. It therefore appears likely that the exposure

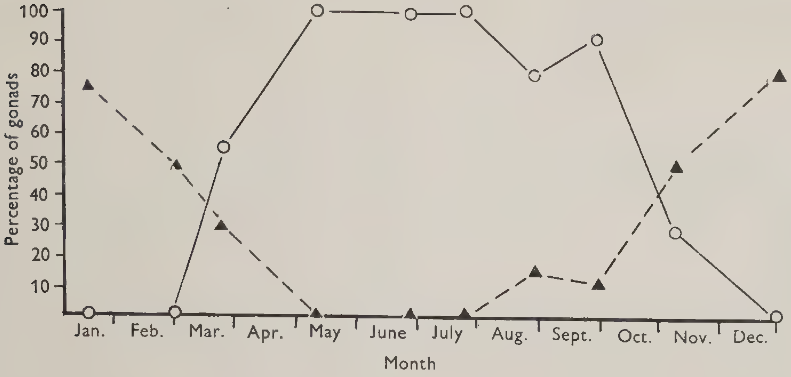

Fig. 10. Histological condition of ovary throughout the year in the Clyde samples. ○—○, percentage of gonads with yolk-laden eggs; ▲—▲, percentage showing phagocytosis of eggs.

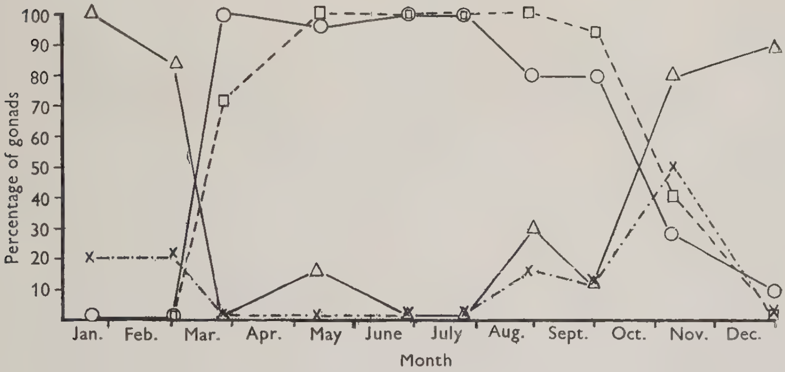

Fig. 11. Histological condition of testis throughout the year in the Clyde samples. ○—○, percentage of gonads with dividing male nuclei; □ - - - □, percentage with sperm; △—△, percentage with phagocytes in peripheral part of testis; × – · – ×, percentage with phagocytes in central part of testis.

TABLE I. EFFECT OF LEVEL ON THE SHORE ON GONAD DEVELOPMENT

		Percentage of gonads with						
Date	Level on shore (ft. above Chart Datum)	Male nuclei dividing	Sperm	Phagocytes in central testis	Phagocytes in peripheral testis	Yolk in eggs	Phagocytosis of eggs	Diameter of largest oocytes (μ)
6 March 1953	2·2	100	58	10	2	30	15	135
	7·1	91	50	7	21	22	30	126
11 May 1953	2·2	100	100	0	0	100	0	279
	7·1	100	100	0	0	96	0	287

to air which the Clyde animals experienced was not the main cause of difference in breeding behaviour between these and the Essex animals.

Phagocytosis in the Gonad

The process of phagocytosis in the gonad following spawning has been recorded only twice in ascidians, to my knowledge. Carlisle (1951) observed the formation of corpora lutea in the ovary of *Ciona intestinalis* (L.) after ovulation had been artificially induced by injection of mammalian gonadotrophin, and Pérès (1952) described phagocytosis in the testis of the same species during sexual inactivity. Carlisle described the invasion of nearly ripe ova by the surrounding follicle cells, which phagocytosed the yolky material, and subsequently liberated a secretion.

In *Dendrodoa grossularia* essentially the same process occurs after natural ovulation. The inner follicle cells, which lie in the protoplasm of the ovum immediately internal to the chorion, migrate inwards, multiply apparently with amitotic division of the nucleus, and start to ingest the yolk granules (Fig. 9, *right*). The chorion and outer follicle cells eventually disappear, and a mass of swollen phagocytes is left which contain the remains of the yolk granules. I have not been able to decide whether a syncytium is formed, such as Carlisle described in *Ciona*, but after multiplication, and ingestion of yolk, the phagocytes are certainly closely packed together. Nor have I seen any clear evidence of the production and liberation of a secretion, although this may be formed.

In the testis, of course, the origin of the phagocytic cells is different. They are probably derived from the blood or connective tissue surrounding the testis, since phagocytes are common elements of these tissues in many ascidians. Their cycle of activity begins with a migration into the tissue of the testis. When they have penetrated to the central part of the lobes where residual sperm are situated, they ingest these sperm and perhaps also spermatids. They then withdraw with their contents to the peripheral part of the testis lobes and eventually disappear, perhaps by moving out of the testis altogether although this was not discovered.

How far the processes involving phagocytosis in the ovary and testis can be regarded as comparable depends on whether the function of the phagocytes in the ovary is primarily to remove unshed eggs, or whether this removal is merely a necessary step towards the production of a secretion. In the testis it appears that no more is involved than the removal of unshed germ cells. Also the origin of the phagocytic cells is different in the two cases. The question cannot be answered until we know something about the nature of the supposed secretion formed in the ovarian part of the gonad.

Factors influencing Breeding

Temperature is probably the most important of the factors influencing the breeding of marine invertebrates. In many species there is no fixed temperature at which spawning occurs, but rather, as Korringa (1941) says of *Ostrea edulis* L., 'the maturation-period of the eggs is a function of time and temperature'. This has been shown to be true, for instance, in *Crassostrea virginica* (Loosanoff & Davis, 1952). Nevertheless, these workers also found that a certain minimum temperature is required before maturation of the gonad can start. Very little is known of the precise effect of other factors, e.g. food, in initiating the reproductive cycle. Once the gametes are mature they may be liberated in different species by a variety of stimuli, of which temperature change is one of the commonest.

When considering the influence of temperature on the breeding of *Dendrodoa grossularia* we must first recognize that the Clyde animals studied, being intertidal in habitat, were subjected to air temperature for varying daily periods. The effect of air temperature on the animals during their period of exposure is unknown. It seems likely, however, to have been much less than the effect of water temperature during submersion, since some of the most important vital activities, e.g. water filtering and feeding, cease during exposure to the air. The evidence from samples taken from points high and low on the shore also suggests that exposure to air was not a major factor affecting the rate of gonad development. It is perhaps reasonable, therefore, to consider only water temperature when dealing with the Clyde animals. Yet it is difficult to find any correlation between the sea-surface temperature and the onset of those changes in the gonad which initiated the breeding cycle. Thus although the water temperature in March was relatively steady between 7·0 and 7·5° C., it was during this period that important and rapid changes were accomplished in both male and female parts of the gonad (Figs. 10, 11). Similar changes in the gonads of the Essex animals had occurred at least a month before (Figs. 7, 8), during a time when the water temperature there rose quickly from 0·0 to 3·5° C. Unless we assume the existence of physiologically differentiated races in the two localities, adapted to different temperature ranges, we must conclude that it was not temperature *level* which determined the onset of those changes in the gonad. Nor was it a rising temperature that started the maturation of the gonad in the Clyde animals, although this could explain the results obtained in the Essex samples.

The first liberations of ripe gametes from the gonads occurred in spring at about the same water temperature, 8·0–9·0° C., in the Essex and Clyde animals. At the approach of winter the liberation of gametes from the gonads ceased when the temperature dropped to about 11·0° C., in both areas.

It is more difficult to discover whether there also exists an upper temperature limit to the liberation of gametes. The answer to this question depends on the explanation adopted for the absence of breeding during July in Essex and the reduced breeding in July and August in the Clyde. Two possible explanations can be considered. The first is that intense spawning in early summer exhausted the animals in Essex so that by July they were spent. If this explanation were right the Clyde animals, having bred with much less intensity, should still have been capable of undiminished breeding thoughout the summer. But in fact there was a definite reduction of breeding in July and August, and the theory of exhaustion is therefore unsatisfactory.

The second possible explanation is that, in addition to the lower temperature limit which has been demonstrated, an upper temperature limit to the liberation of gametes from the gonad also exists. Histological examination shows that in July the gonads of the Essex animals still had large yolky eggs but these, instead of being shed, were retained and attacked by phagocytes, a process suggesting that an environmental factor suppressed further ovulation. That factor may well have been high temperature.

It appears that, at temperatures over 15·0° C., ovulation becomes progressively less, and above about 20·0 ° C. is totally suppressed.

The present investigation has raised a number of problems concerning the influence of environmental factors on breeding which can probably only be solved by experiment.

SUMMARY

Samples of the ascidian *Dendrodoa grossularia* were studied from a sublittoral habitat in the River Crouch, Essex, and from a littoral habitat on the Isle of Cumbrae, Firth of Clyde. The pattern of annual growth was similar in these areas, and the life-span appeared to be 18 months to 2 years.

Developing eggs are retained in the atrial cavity by brood filaments.

In Essex a first maximum of breeding in spring and early summer was separated from a second maximum in late summer and autumn by a short interval when no breeding occurred. In the Clyde breeding was continuous from early summer to autumn, but was slightly reduced in August.

The histological changes in the gonad are described. Phagocytosis in both ovary and testis followed each period of breeding.

Gametes were shed from the gonad when the sea temperature in spring reached 8·0–9·0° C., and ceased to be shed in autumn when the sea temperature fell below about 11·0° C. Release of gametes appeared to be reduced at temperatures above 15·0° C. and totally suppressed above about 20·0° C.

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J. Mar. biol. Ass. U.K. (1954) 33, 49–60
Printed in Great Britain

CONCENTRATION GRADIENTS AND THEIR SIGNIFICANCE IN *LAMINARIA SACCHARINA* (L.) LAMOUR.

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(Text-figs. 1–5)

In previous publications the author (Black, 1948*a*–*c*, 1949, 1950*a*) has reported the seasonal variations in chemical composition that occur in certain of the common Phaeophyceae. In the sublittoral and littoral zones it has been found that a correlation exists between the chemical composition and the depth of immersion of the plant (Black, 1950*b*, and unpublished work). Differences in composition, particularly during the summer months, also occur in the same species taken at the same time and depth but from different habitats (Black, *loc. cit.*), and there is also evidence that in the laminarians the stage of development of the plant has a profound influence on the composition. In all the above-mentioned analyses the entire plants have been separated into stipe and frond, dried under controlled conditions, milled, and a well-mixed representative sample of each taken for analysis.

Work on the distribution of iodine and potassium in *Laminaria flexicaulis* (=*digitata*) has been carried out by Spindler (1948), Rinck (1948), Rinck & Brouardel (1949*a*, *b*), and Brouardel & Rinck (1950). Rinck & Brouardel (1949*a*) determined the iodine content of 325 samples of the fresh and dried alga, and found it to vary regularly with the distance along the frond, being at a minimum at the base of the frond where the youngest cells are and then increasing with the length of the frond. A similar variation was also obtained in the water content of different parts of the frond. As with iodine, they found a regular variation in the potassium content, the results indicating a direct relation between the morphological portions of the alga—holdfast, stipe, stipe-frondal zone and frond—and the concentration of the chemical elements. Moss (1948, 1950), in her studies on the structure and chemical composition of *Fucus vesiculosus*, has shown that a concentration gradient exists in the thallus and that the receptacles and sterile tips differ markedly in composition. As anatomical differentiation proceeded from the apex, the region of growth, to the base of the thallus, so did the chemical composition vary: the percentage dry weight and organic nitrogen (expressed as percentage fresh weight) increased while alginic acid decreased.

Recent work by Gerdes (1951) on the distribution of aneurin in the algae has shown a constant decrease in the aneurin content with distance from the growing point. Sections of *Laminaria saccharina* were analysed from the transition zone, the upper part of the frond, the stipe and the holdfast. The transition zone contained 7·25 p.p.m. and the upper part of the frond 1·31 p.p.m. (dry basis).

Observations on the rate of growth of *L. digitata* and *L. saccharina* in the Dalne-Zelenetzky Gulf were made by Kuznetzov (1946), and Parke (1948) has shown that in *L. saccharina*, from the coasts of Devon and Argyll, there are two periods of growth, (i) a period of rapid growth from January to June/July with the most rapid growth between March/June, and (ii) a period of slow growth from July to December with the slowest period between September and December. Throughout a yearly cycle regular changes in growth rate affected the length, width, and thickness of the frond, while loss of frond tissue from the distal end started when a plant was a few months old and continued throughout its life. According to Parke (1948) there can be present in a *L. saccharina* frond in October a portion of newly formed tissue and tissue at least 7 months old. It is logical, therefore, to expect variations in composition with age. As the stipe of this species is relatively small compared to the frond, and as no variation existed in the dry-matter content along the stipe, only the frond was in each case analysed.

No work appears to have been carried out to show the presence of a concentration gradient in *Laminaria*. Moss (1948, 1950) has studied *Fucus vesiculosus*, but, whereas in *Fucus* the growing region is near the tips of the branches, in *Laminaria* it is at the base of the frond.

This work forms part of a programme of research and development of the Institute of Seaweed Research to whom the writer is indebted for permission to publish. The writer is also indebted to Dr E. T. Dewar for his great care in collecting and sectioning the plants, to W. J. Cornhill for his assistance with the analytical work and to F. T. Walker for his advice and encouragement. An abstract of this paper was read at the 2nd International Biochemical Congress in Paris in July 1952.

PREPARATION OF SAMPLES AND METHODS OF ANALYSIS

For the investigation now to be reported samples were collected and treated as follows.

The initial sample of *L. saccharina* (L.) Lamour. was collected on 19 August 1949 at Shandon in the Gareloch at 4 m. (low water). This was plant 1, which had the dimensions shown in Fig. 1 A. The frond was divided transversely into five equal sections 30·5 cm. long and dried at 25–30° C. for 24 hr.

Plants 2–4 were collected in Kerrera Sound, Oban, Argyllshire, at approximately 4 m. (low water), on the 17 October 1950, 26 April 1951 and 30 May 1951 respectively, and sectioned as shown in Fig. 1. The sections were dried at 100° C. for 16 hr. Each of the dried sections was then milled in a Christy and Norris no. 8 Laboratory Mill fitted with a 64-mesh screen, and analysed as previously described (Black, 1948*a*; Black & Cornhill, 1951).

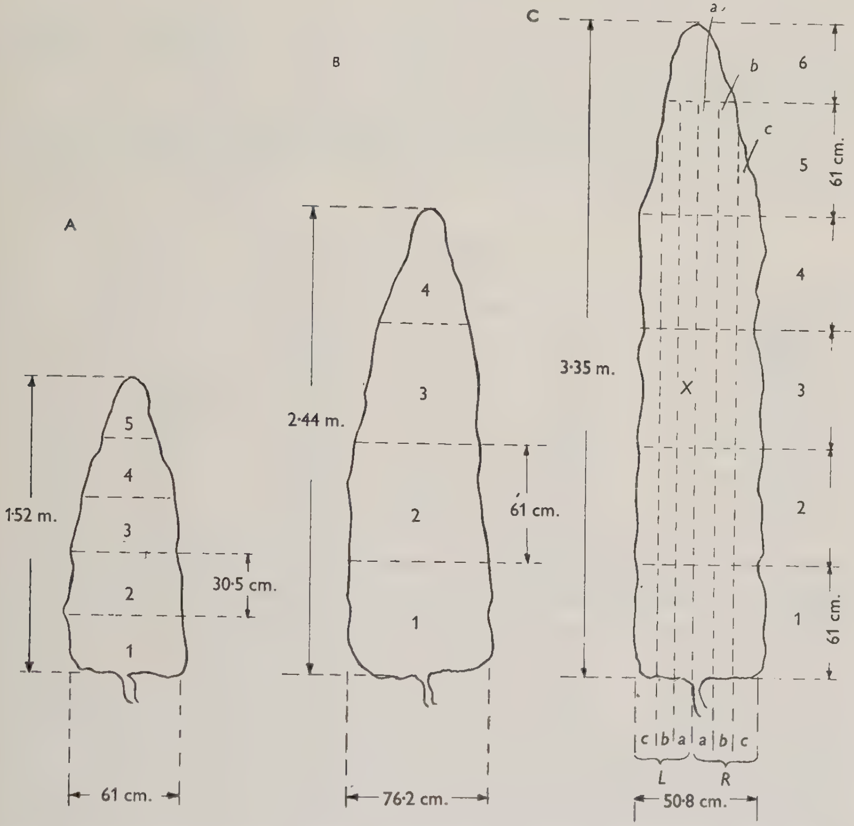

Fig. 1. Method of dividing the fronds investigated. A, plant 1, Gareloch, 19 August 1949. B, plants 3 and 4, Kerrera Sound, 26 April 1951 and 30 May 1951. C, plant 2, Kerrera Sound, 17 October 1950. All were divided into transverse 'sections' numbered from the base. In A there were five sections of 30·5 cm.; in B, four of 61 cm.; in C, six sections of which the first five were 61 cm. long and the terminal 30·5 cm. In C there were, in addition, longitudinal divisions. The frond was first divided into right and left halves (R and L). Each half was divided further into three strips, *a*, *b* and *c*, the first the innermost. $a = b = 7{\cdot}6$ cm., $c = 10{\cdot}2$ cm. (Any of the 30-odd subdivisions of the frond can be specified by a combination of three symbols: thus the unit marked *X* would be called L 3*a*.)

RESULTS

The results obtained are expressed as fresh-weight and dry-weight percentages. When calculated as percentage concentrations in the water present the graphs were parallel to those for the fresh weight and have been omitted.

The calculation of the results on the 'residual dry weight' was also considered, but, as this underwent considerable variation, calculation on the fresh weight, which approximately gives the results in terms of concentration in the water present, was preferred.

Plant 1

The figures for cellulose, laminarin, alginic acid, crude proteins, total ash and mannitol in plant 1 are given in Table I.

The results show that in the section of frond adjacent to the stipe, (1 in Fig. 1 A), which is the growing region of the plant, the mannitol and ash contents are high, the crude protein content is low, and laminarin is absent.

TABLE I. PLANT 1. PERCENTAGE COMPOSITION (DRY BASIS)

Percentage on dry basis	Section of frond				
	1	2	3	4	5
Total ash	28·8	26·4	19·4	20·0	26·0
Mannitol	29·5	30·6	8·8	9·6	10·6
Laminarin	1·0	4·0	16·4	22·0	12·4
Crude proteins	15·4	16·8	20·5	19·6	18·2
Alginic acid	8·4	13·4	13·4	18·3	17·7
Cellulose	3·8	3·8	3·8	3·8	3·8
Iodine	0·65	0·49	0·47	0·42	0·40
Fucosterol	0·10	0·06	0·06	—	—

In section 2, with the exception of the ash, slight increases occur in the content of the other constituents, while in section 3 marked differences occur, laminarin increasing from 4 to 16·4 %, crude proteins from 16·8 to 20·5 % with a correspondingly large decrease in mannitol from 20·5 to 8·8 % and ash from 26·4 to 19·4 %. Section 4 shows the laminarin still increasing, while there are no significant changes in the ash, crude proteins and mannitol contents. In section 5 a marked decrease in the laminarin content occurs with a correspondingly large increase in the ash content.

Iodine was found to decrease from 0·65 % in section 1 to 0·49 % in 2, 0·47 % in 3, 0·42 % in 4 and 0·40 % in 5 (dry basis), while fucosterol decreased from 0·10 % in section 1 to 0·06 % in section 2, quite a significant difference for this sterol. The alginic acid content increased from 8 % in section 1 to 18 % in sections 4 and 5.

Plant 2

These results having shown that marked differences in composition occurred along the frond, a more detailed investigation was carried out with plant 2 which was divided into thirty-one areas (Fig. 1 C), one at the tip, with the five transverse sections subdivided each into six longitudinally. The frond being thickened medially the innermost sections were about twice as heavy per unit area as the outer with the percentage water only slightly higher in the central strip.

Dry Matter

As one passes up the axis of the frond there is a marked increase in dry matter (Fig. 2). This reaches a maximum two-thirds of the way up and then decreases towards the eroded tip. It is interesting that although the dry-matter content increases, e.g. from 12·6% in L 1*a* to 31·1% in L 4*a* the fresh weights of the sections remain practically constant (45·5 and 47·0 g respectively). Also sections L 1*a* and R 1*a* contain double the amount of dry matter of the adjoining sections L 1*b* and R 1*b*, but the same percentage dry weight, indicating that they have approximately the same percentage composition but differ in thickness. No significant difference in concentration occurs across the frond.

Mineral Matter

The figures for the mineral matter (total ash) expressed on the dry basis, and on the fresh-weight basis, are given in Figs. 2 and 3. When expressed on the dry basis (Fig. 2) the mineral matter is high in the sections adjoining the stipe (25–35%), then decreases half-way up the frond (19–23%), and then increases slightly. This is also true if the ash is calculated on the 'residual dry-weight' basis. On the fresh basis (Fig. 3) almost the reverse occurs, the mineral matter increasing two-thirds of the way up the frond and then decreasing towards the tip. The mineral matter, therefore, increases as the dry-matter content increases and as the mannitol decreases. Possibly mannitol is being utilized in respiration or for further synthesis and mineral matter is diffusing into the cells to control the osmotic pressure. The concentration of mineral matter across the frond is somewhat irregular.

Mannitol

Although mannitol is unlikely to be the primary product of photosynthesis the value found is usually regarded as indicative of the amount of photosynthesis. In the sections adjacent to the stipe (Fig. 3) the mannitol content is high but increases to a maximum one-third of the way up the frond then decreases towards the tip, falling from 35% to 11·5% of the dry matter.

Laminarin

Laminarin is regarded as a storage foodstuff. It is absent from the stipe of the laminarians and from the frond during the period of rapid growth in the spring. It appears to be formed during the period of slow growth when the nitrate and phosphate content of our inshore waters is very low. Laminarin is almost entirely absent from plants growing in habitats where the above nutrients are available all the year as a result of pollution or upwelling. The results for this carbohydrate are given in Fig. 4. Laminarin is entirely absent from the active growing sections adjacent to the stipe, and increases up the frond to 30·4% in L 4*c* and 34·5% in R 3*b*; in general it increases with the dry-matter content and then decreases to 5% at the tip.

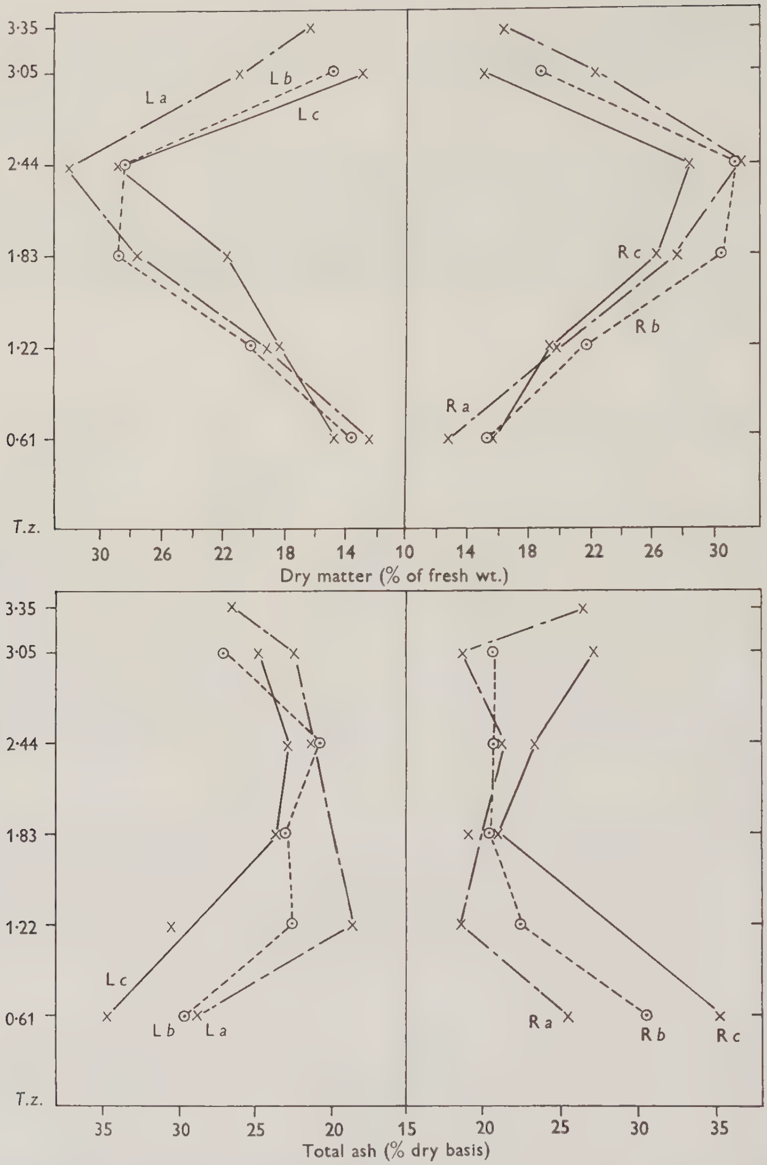

Fig. 2. Plant 2. Variation in (above) *dry-matter content* as percentage of fresh weight, and (below) *total ash* as percentage of dry matter. Each graph line represents one strip of the frond, with one value entered for each section. Left and right sides are graphed separately. *T.z.*, transitional zone at base of frond. Vertical scale *d* represents distance along the frond, from the base, in metres.

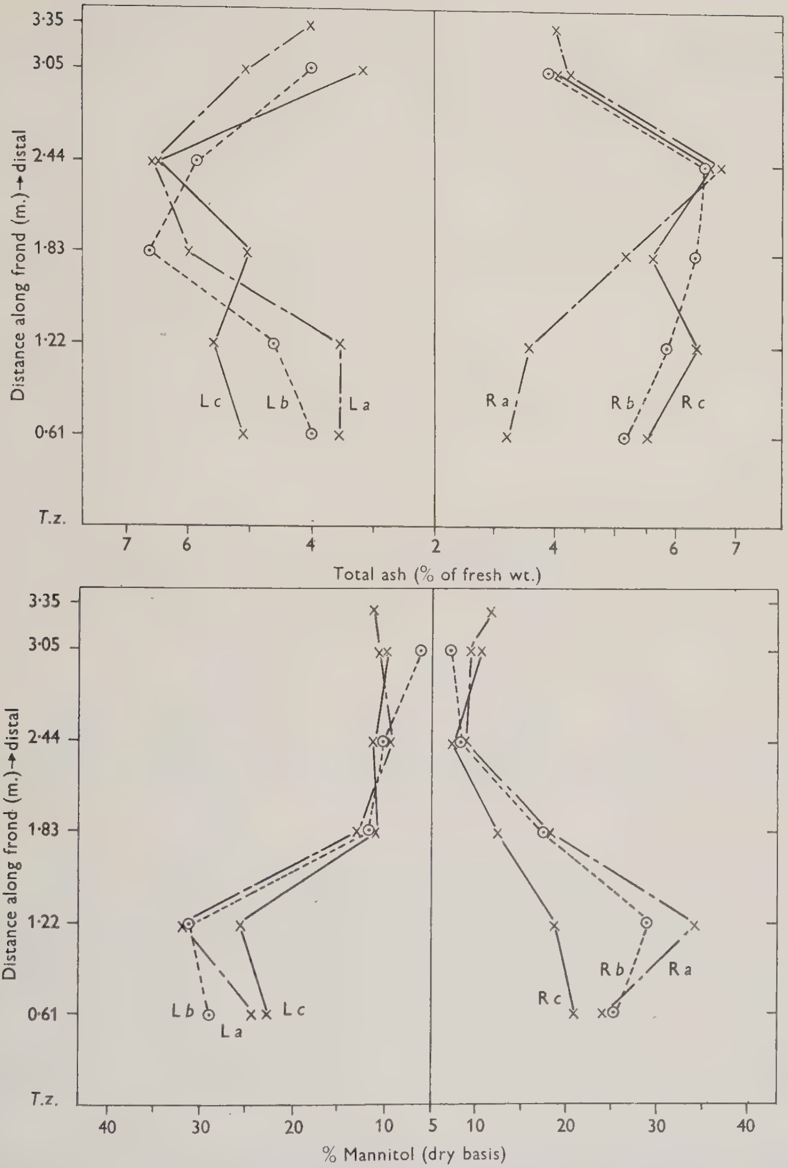

Fig. 3. Plant 2. Variation in (above) *total ash* as percentage of fresh weight, and (below) *mannitol* as percentage of dry matter. Particulars as in Fig. 2.

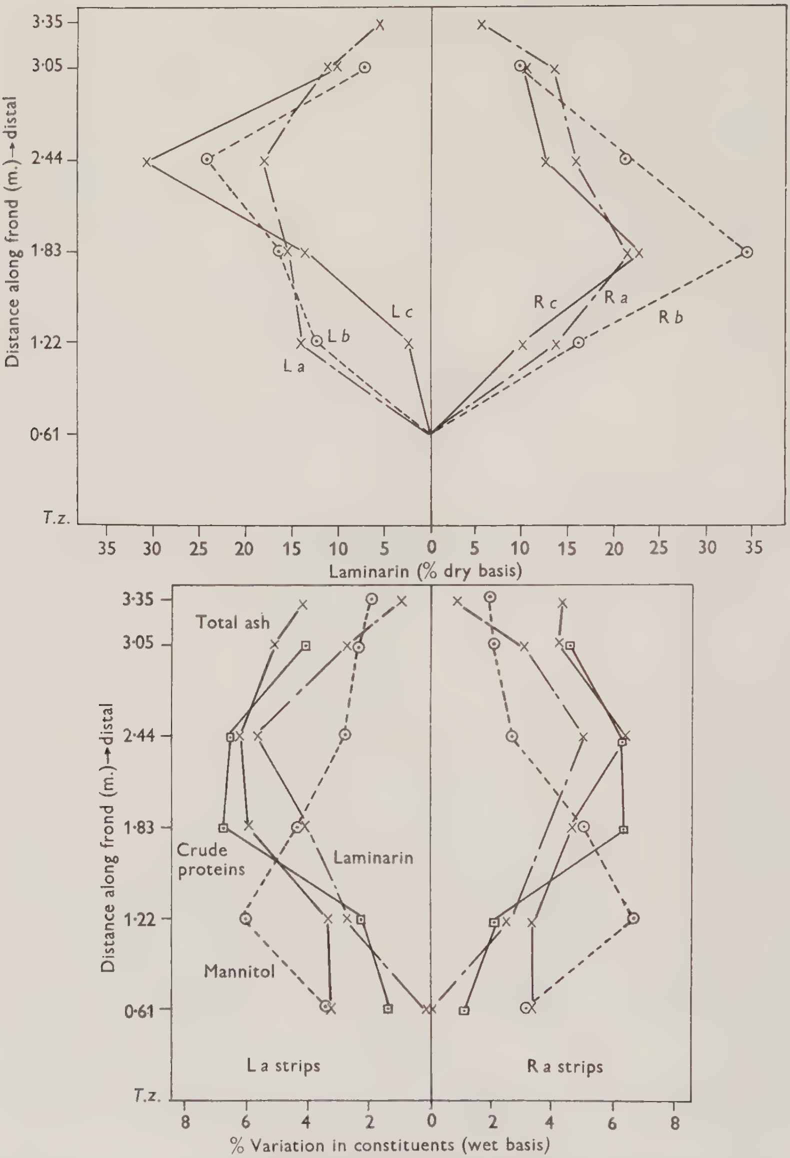

Fig. 4. Plant 2. Above: variation in *laminarin* as percentage of dry matter. Below: percentage variation in four constituents compared, on a wet basis, only the *a* strips considered.

Alginic Acid

The alginic acid content is exceedingly low in the sections L 1*a* and L 2*a* and is of such a low grade (i.e. of low molecular weight) that it is indeterminable by the standard method of analysis. In L 3*a* and L 4*a* values of 13·6 and 13·1 % were obtained, indicating that a higher polymer of alginic acid is no doubt present.

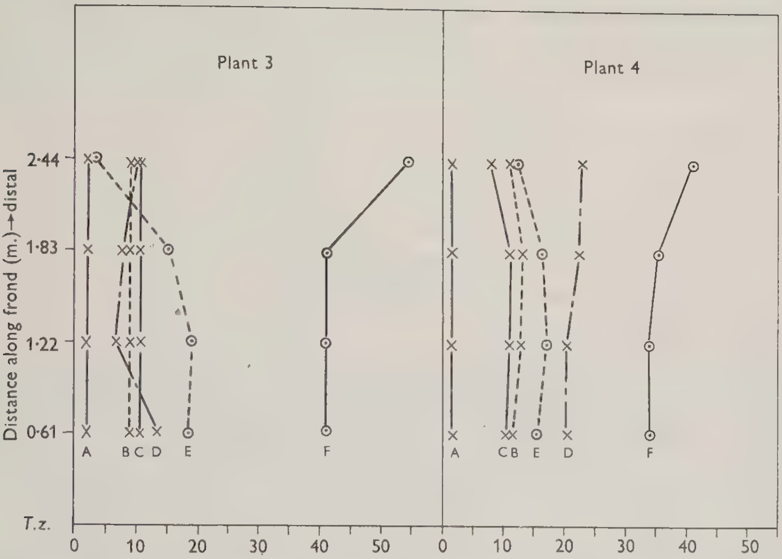

Fig. 5. Plants 3 and 4, illustrating the period of rapid growth. Variation in (A) *laminarin*, (C) *crude protein*, (D) *alginic acid*, (E) *mannitol*, (F) *total ash* (on dry basis); and (B) *dry matter* (on wet basis)

Plants 3 *and* 4

In order to prove that variations such as those recorded above were improbable during the period of rapid growth in the spring, plants 3 and 4 were taken and sectioned as in Fig. 1 B, and the analytical results are given in Fig. 5. The graphs show clearly that at this time of the year the composition of the frond is constant throughout its length, only at the tip where erosion takes place is there any noticeable change.

In previous publications (Black, 1948*b*, 1950*a*) the composition of the frond of *L. saccharina* is given for a period of 4 years, and the results agree with those of the present investigation. Plants 3 and 4, taken in April and May during the period of rapid growth, are composed mainly of tissue laid down during the preceding 3 months with perhaps only the distal part retained from the previous year, whereas plants 1 and 2 are composed of new tissue adjacent to the stipe and tissue up to 7–8 months old.

Analysis of a frond of *L. cloustoni* in May, when it was possible to differentiate between the new and old growth, showed that the old frond was low in mannitol but still contained laminarin, while the new frond was appreciably higher in mannitol but contained no laminarin. In April/May the composition of an *L. saccharina* frond will, therefore, depend on whether old growth from the previous year has been retained or cast. From May onwards loss of frond is then due to erosion at the tip.

In Fig. 4 the graphs for the sections representing the central portion (L*a* and R*a*) have been superimposed and give a general picture of the variations in composition up the frond. In this case the results are all expressed on the wet basis, which is believed to give a more accurate picture of the living plant.

Discussion of Results

Four fronds of *L. saccharina*, collected at different periods of the year, have been sectioned and analysed. The two fronds taken in April/May during the period of rapid growth are composed chiefly of new tissue and there is little difference in composition along the length of the frond except at the tips where erosion has occurred.

The plant collected in August, during the period of slow growth, showed considerable variations in composition due to the presence of tissue of different ages. This was confirmed in the plant collected in October, the frond of which was cut into thirty-one sections which were weighed separately and later dried and analysed for dry matter, total ash, mannitol, laminarin, alginic acid and crude proteins. In the active growing region which is proximal to the stipe, where cells are dividing to form new tissue, and practically along the whole length of the frond during the period of rapid elongation, laminarin is absent and the dry-matter content is very low (10–12 %) and is composed chiefly of mineral matter (36–40 %) and mannitol (20–30 %). In the active growing region the proteins are at a minimum, and the alginic acid is of such a low grade as to be indeterminable by the standard method. The results are in good agreement with those of Moss (1948) who found the dry matter and inorganic nitrogen, for example, lowest, and the mineral matter highest in the distal samples (tips) of *Fucus vesiculosus* where the growth is apical.

During the period of rapid growth there is extensive elongation due to cell enlargement with vacuolation. Then the tissue is low in dry matter composed mainly of mannitol and mineral matter and laminarin is absent. In July (Parke, 1948) a period of slow growth commences with the slowest growth between September and December when plants 1 and 2 were collected. June to September is also the period (Black & Dewar, 1949) when the nitrate and phosphate content of our inshore waters is at a minimum. During the period of slow growth, therefore, the stipo-frondal zone would alone represent new cells while the remainder of the frond, although able to photosynthesize, ceases active cell division and accumulates carbohydrates fated to become the food reserve for the winter or for sporogenesis. What Parke described as mature tissue is, therefore, the storage portion of the frond two-thirds of the way up and containing a reserve of laminarin with proteins; the distal portions represent tissue which has spored or is badly eroded. Iodine and fucosterol are highest in the stipo-frondal (or transition) zone where they are in all probability required for new growth.

The results show that at certain times of the year concentration gradients of carbohydrates, proteins and mineral matter exist up the frond; they may be the result of restricted growth and may not be present in plants from habitats where nutrients are available all the year round and where laminarin has already been shown to be absent (Black, 1950*a*).

There is no evidence of a concentration gradient across the frond although the thickness shows considerable variation.

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J. Mar. biol. Ass. U.K. (1954) 33, 61–63
Printed in Great Britain

ON THE HORMONAL INHIBITION OF MOULTING IN DECAPOD CRUSTACEA

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Recently I have reported experiments which have led me to throw doubt on the universal applicability of the theory of the production of a moult-inhibiting hormone by the eyestalks of the decapod Crustacea (Carlisle, 1953). I suggested that the eyestalk moult-inhibiting hormone might be associated with the existence of a definite moulting season. In animals which possessed such a moulting season moulting would then be inhibited outside this season, but within the season there might well be no such inhibition. The experiments reported here support a modified form of this hypothesis.

Small crabs of the species *Carcinides maenas* (Pennant) were used. All specimens were between 2·5 and 4 cm. in carapace breadth. The sexes were not separated but no females were in berry. The crabs were kept individually in glass jars (4 lb. rock jars or Breffitt jars) of about 3 l. capacity, in 1 l. of sea water. The temperature was 18·4 ± 1·5° C. The animals were fed once a week on squid flesh and the water changed after feeding. A group of about 250 crabs was selected and divided at random into two groups. In group E (experimental) the left eyestalk was severed at the base and cauterized. In group C (control) the retinal portion of the left eyestalk was destroyed by cautery. Two days later the right eyestalks of group E were removed and the right eyes of group C seared to destroy the retinal portion. The animals which survived these operations (eighty-four of group E and eighty of group C) were then isolated in the Breffitt jars. During the next 49 days six crabs moulted in each group. On the fiftieth day the blood calcium level of the survivors which had not moulted was measured (see Table 1). The death-rate had been almost identical in the two groups.

Four crabs in group C and five in group E showed signs of an approaching moult in the heightened blood calcium level. Except for these nine crabs, in which the blood calcium level was of the order of 100 mg. %, the level ranged between 28 and 41 mg. % in both groups. The mean for group E (excluding the five very high ones) was 33·4 mg. % and for group C 35·2 mg. %. Quite clearly the two groups do not differ in this respect and neither operation precipitated moulting nor even initiated proecdysis, for then the blood calcium level would be enhanced after a full 50 days, whereas this has only happened in a few animals which doubtless had commenced proecdysis without the extraneous stimulus of the operation.

Histological examination showed that the crabs of group C had intact the sinus gland and X organ, which were of course absent in the animals of group E. Now the moult-inhibiting hormone of crabs has been shown to emanate from the X organ-sinus gland complex (e.g. Passano, 1953). Under the experimental conditions I have employed, then, destruction of the seat of the eyestalk moult-inhibiting hormone has not precipitated moulting nor initiated proecdysis. Evidently, then, under these conditions no moult-inhibiting hormone from the eyestalk is interfering with the initiation of proecdysis.

Now according to Broekhuysen (1936) *C. maenas* moults seasonally. It does not moult during the months of February, March and April. This experiment started at the end of May and continued through all June and part of July.

TABLE I. BLOOD CALCIUM LEVELS IN THE BLOOD OF CRABS 50 DAYS AFTER THE BEGINNING OF THE EXPERIMENT

(Expressed in mg./100 g.)

Group E	Group C	Group E	Group C
35·0	41·6	38·7	39·6
31·8	35·2	32·1	39·2
46·8	34·4	30·4	35·6
30·1	32·5	23·2	29·9
29·2	31·8	38·4	35·9
27·4	28·6	38·6	39·8
38·5	36·8		
30·3	38·2	100·7	84·3
32·2	37·5	96·3	94·2
32·7	36·5	109·8	100·3
34·4	36·8	87·4	105·6
32·5	26·4	93·7	

That is to say it lay wholly within the moulting season. This is the chief difference from the experiments of American workers who have experimented almost entirely on animals outside the moulting season, when they have found clear evidence of the existence of a moult-inhibiting hormone secreted by the X organ-sinus gland complex. This is true of *Carcinides* (Passano, personal communication). But within the moulting season I have found no evidence of a moult-inhibiting hormone produced by the eyestalk. That some factor is inhibiting moulting there can be no doubt, for most of the animals in both experimental groups show blood calcium levels characteristic of anecdysis. Stephens (1951) has produced evidence that a moult-inhibiting hormone is produced in the central nervous system. It is possible that this is the factor responsible for the inhibition of moulting during the moulting season in those species which during this period do not allow one metecdysis to run straight into the next proecdysis.

The conclusion seems to be that whereas outside the moulting season ecdysis may be inhibited by a hormone secreted by the X organ-sinus gland complex of the eyestalk, and removal of the eyestalk or of this complex leads to the initiation of proecdysis, in the moulting season removal of the eyestalk has no such effect and there is no evidence of the secretion of a moult-inhibiting hormone by the eyestalk. Moulting may, however, be inhibited during this period by some factor which emanates from some other centre than the eyestalk.

My thanks are due to my friend Dr P. F. R. Dohrn, of the Naples Zoological Station, in collaboration with whom I began investigations into the problem of moulting in Crustacea and who has been unfailing in his help and encouragement.

SUMMARY

During the moulting season in *Carcinides maenas* ablation of the eyestalks does not initiate proecdysis. Blood calcium levels, however, indicate that during this period moulting is partially inhibited by some factor emanating from some other centre than the eyestalk.

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J. Mar. biol. Ass. U.K. (1954) 33, 65–68
Printed in Great Britain

THE EFFECT OF MAMMALIAN LACTOGENIC HORMONE ON LOWER CHORDATES

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If a male dogfish is held in the hand and its belly stroked firmly backwards with the ball of the thumb a fluid can be expressed from the cloaca. In my experience this fluid in a fresh animal invariably contains sperm, whatever the season of the year. After a period of inanition, however, sperm eventually disappears from the cloacal fluid. Such an animal is a convenient test object for gonadotropic activity by a method which closely parallels the toad-pregnancy test of Galli Mainini (1947).

In a former paper I have shown that ascidians respond to an injection of chorionic gonadotropin by ovulation and release of sperm into the water (Carlisle, 1951). Anterior lobe pituitary gonadotropic extract (Praephyson) has the same effect in equivalent doses, when tested on *Ciona intestinalis*.

Materials used

The anterior lobe gonadotropic hormone used was the German Praephyson (Chemische Fabrik Promonta, Hamburg), stated to be assayed at 15 m.u. luteinizing hormone per ml. Reassayed by me on mice by the Ascheim-Zondek test it titred 70 i.u./ml.

Chorionic gonadotropin was extracted from human pregnancy urine by Dr W. M. S. Russell and myself, and assayed by the Ascheim-Zondek test and by the *Xenopus* ovulation test at 110 i.u./mg. This material was dissolved immediately before use at the rate of 1 mg./ml. of distilled water, yielding a solution containing 110 i.u./ml.

The lactogenic hormone was extracted from human *post-partum* urine. Urine was taken 1–7 days *post partum*, at which time there is the highest content of lactogenic hormone. After adjusting the pH to approximately the isoelectric point of the hormone (pH *c.* 5·6) 5% by volume of a saturated solution of benzoic acid in acetone was added slowly with stirring. Microcrystals of benzoic acid are thus formed which supply a large surface for the adsorption of the hormone and other proteins. The mixture was left in a refrigerator overnight. The benzoic acid with its adsorbed proteins was then filtered off. The filtrate was then re-extracted in the same way. The combined fractions of benzoic acid were washed with distilled water and then the benzoic acid washed out with several changes of absolute acetone, in which the proteins are insoluble. The proteins are thus left behind as a precipitate, free of benzoic acid. The precipitate is dissolved in 50% acetone, and any remaining insoluble material discarded. The dissolved material was precipitated by raising the acetone concentration above 92% and filtered off.

This precipitate was then purified and crystallized by the dilute acetone method of White, Bonsnes & Long (1942).

This method of crystallization has apparently failed in the hands of a number of workers. The method depends, in the last analysis, on the isoelectric precipitation of the hormone, with the precipitation delayed by the addition of acetone to give time for crystals to form, instead of a fast amorphous precipitation. Obviously, then, the final solution must be at the isoelectric point of the hormone at the time of precipitation. In the outline of the method given by White *et al.* the adjustment of the solution to the isoelectric point before the addition of acetone is prescribed, but no mention is made of the necessity of readjusting the pH after the addition of acetone. Acetone, even of Analar grade, frequently departs quite widely from the desired pH, and correspondingly the solution to which this acetone is added is altered in pH away from the isoelectric point. Under these conditions crystallization, or indeed precipitation, is unlikely to occur. It is thus essential to readjust the pH of the mother liquor to the isoelectric point of the hormone after the addition of acetone.

The yield of crystalline lactogenic hormone by this method is approximately 20 mg/l. of urine. It assays at about 25 i.u./mg. as tested by the pigeon-crop method. When tested by the Ascheim-Zondek test for gonadotropic content, 10 mg. administered to each of five mice gave no response. Ten i.u. of chorionic gonadotropin (0·09 mg.) produced a positive response in a parallel test.

Experimental data

An injection of 1 or 2 mg. of chorionic gonadotropin dissolved in distilled water into starved male dogfish—four tests at each level of dosage—stimulated a reappearance of sperm in the cloacal fluid. An injection of 0·5 mg. had no such effect on six animals. Presumably this was below the threshold of activity.

An injection of 2 mg. of lactogenic hormone dissolved in distilled water into each of seven animals stimulated the reappearance of sperm in the cloacal fluid.

An injection of 1 ml. distilled water into each of seven animals had no effect.

An injection of 1·5 ml. praephyson into each of six animals stimulated the reappearance of sperm in the cloacal fluid.

An injection of 0·5 ml. praephyson into each of six animals had no effect.

An injection of 1 mg. of chorionic gonadotropin into each of ten *Ciona* provoked ovulation and sperm discharge. An injection of 1 ml. praephyson into each of five *Ciona* provoked ovulation and sperm discharge.

An injection of 1 mg. lactogenic hormone into each of ten *Ciona* provoked ovulation and sperm discharge.

An injection of 0·1 mg. chorionic gonadotropin into each of ten *Ciona* had no effect.

An injection of 1 ml. distilled water into each of ten *Ciona* had no effect.

The results of these tests are listed in Table I. The positive effect in dogfish was as already stated, that in *Ciona* was ovulation and sperm discharge.

TABLE I

(C.G. = chorionic gonadotropin, L.T.H. = lactogenic hormone, each dissolved in distilled water.)

Injection				
Substance	Dose	Subject	No. of individuals	Effect
C.G.	1–2 mg.	Dogfish, ♂	7	+
C.G.	0·5 mg.	Dogfish, ♂	6	0
L.T.H.	2 mg.	Dogfish, ♂	7	+
Distilled water	1 ml.	Dogfish, ♂	7	0
Praephyson	1·5 ml.	Dogfish, ♂	6	+
Praephyson	0·5 ml.	Dogfish, ♂	6	0
C.G.	1 mg.	*Ciona*	10	+
Praephyson	1 ml.	*Ciona*	5	+
L.T.H.	1 mg.	*Ciona*	10	+
C.G.	0·1 mg.	*Ciona*	10	0
Distilled water	1 ml.	*Ciona*	10	0

Comment

Since 10 mg. lactogenic hormone had less gonadotropic effect on mammals than 0·09 mg. chorionic gonadotropin, and since 0·5 mg. chorionic gonadotropin was below the threshold dosage for the dogfish, then the spermatic activity of the lactogenic hormone on the dogfish could not be due to an impurity of gonadotropin contained in the lactogenic hormone, for the assay on mice indicated that 1 mg. of lactogenic hormone must contain less than 0·009 mg. (1 i.u.) of gonadotropic hormone. A similar argument applies to the comparative dosages on *Ciona*. Evidently, then, it must be the lactogenic hormone itself which has had the gonadotropic effect on these two animals. Physiologically speaking, they seem unable to distinguish between the two hormones and respond, apparently, to both of them in the same way. Are we here perhaps at an evolutionary level before the differentiation of a primitive sexual hormone of the pituitary into several different hormones with different functions?

This work was performed in the laboratories of the Department of Comparative Anatomy and Zoology, Oxford, and of the Zoological Station at Naples, while holding a Beit Memorial Fellowship for Medical Research, the Oxford Naples Scholarship, a Senior Demyship of Magdalen College, Oxford, and grants from the Browne Fund of the Royal Society, at various times. I wish to thank Prof. A. C. Hardy, F.R.S., Prof. R. Dohrn, Dr A. E. Needham and Dr W. M. S. Russell.

SUMMARY

Lactogenic hormone of mammals has a gonadotropic effect on dogfish and on *Ciona*, an ascidian. This activity resides in the hormone itself and not in any impurity.

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J. Mar. biol. Ass. U.K. (1954), 33, 69–95
Printed in Great Britain

THE GROWTH RATE OF THE HAKE, *MERLUCCIUS MERLUCCIUS* (L.), IN THE CLYDE AND OTHER SCOTTISH SEA AREAS

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(Plate I and Text-figs. 1–13)

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INTRODUCTION

Hake belonging to the genus *Merluccius* are large fish often growing to over a metre in length; they are predators, being well streamlined and armed with formidable teeth; they feed pelagically on smaller fish of their own and other species. Of the various species distributed round the ocean basins, the European Hake, *M. merluccius* (L.), ranges from Lofoten on the coast of Norway, to Dakar on the African west coast; it is found most plentifully along the edge of the continental shelf of Western Europe but also extends into the Mediterranean and North Sea, while stragglers occasionally range as far as Iceland.

In the Clyde area the hake is common in deep water, being found on the east side of Arran, in Kilbrennan Sound, and in Loch Fyne as far up as Inveraray. Elsewhere, in shallower water, it is less frequent and only smaller specimens are taken. The main fishery for hake is in the area off Tarbert, where they are caught on long lines set in mid water.

The most important papers on the biology of the hake are those of Hickling (1927–33), who made an intensive study primarily of hake from S.W. Ireland, and Belloc (1923, 1929), who worked on the French and North African coasts, while Hart (1948) has published a review in which he has summarized a number of scattered works on the distribution and biology of the species. The present paper describes the results of an investigation on the growth rate of hake in the Clyde area.

METHODS AND GEAR

Since June 1949 trawl hauls have been taken at irregular intervals in the Clyde area from M.F.V. *Calanus*, and the lengths of the hake obtained have been measured to the nearest centimetre below. Most of the hauls have been made in the vicinity of Millport, the majority being taken between Great Cumbrae and Bute. Other hauls have been made farther afield whenever the opportunity arose, and an effort has been made to obtain fish from as many parts of the Clyde area as possible.

The gear used has been of two main types; a small-mesh cotton trawl and a larger-mesh commercial trawl. The specifications of the former have remained relatively constant over the sampling period, whereas those of the large-mesh trawl have changed very considerably. Not only have the proportions and absolute sizes of the various parts changed, but also the mesh size, length of bridles, and size of otter boards have all changed. For this reason no emphasis can be placed on the quantitative aspect of the catches obtained. However, there is strong evidence which suggests that for the size-frequency distribution of the fish, the catches are reliable samples of the population irrespective of the gear used (see p. 74). The sampling of small hake was seriously prejudiced by the loss of the first small-mesh trawl on 10 January 1950, and hake were not again caught with this kind of gear until 15 September 1950.

THE MATERIAL

Between June 1949 and March 1953, 2250 hake were caught on sixty-five occasions. Details of the hauls are given in Table I and are separated into six populations on the basis of their length distributions.

Population *a* has only been caught during the winter of 1952–53, and consists of forty-six fish believed to have been hatched in 1952.

Population *b* consists of ninety-four fish believed to have been hatched in 1951.

Population *c* first appeared in September 1951 when one fish of 30 cm. was caught. Altogether only fifty-six hake of this population have been caught.

Population *d* is the most important group and has provided the majority of the fish, 1551 having been caught during the sampling period. These fish,

TABLE I. HAKE POPULATIONS *a–f*, CAUGHT IN THE CLYDE AREA FROM JULY 1949 TO FEBRUARY 1953

Date	No. in sample	Mean length	Length range	Standard deviation	Position of haul	Gear
				Population a		
1952						
18. xii.	40	10·0	7–14	1·99	Tarbert	Large mesh
1953						
11. ii.	5	13·0	12–14	—	E. side of Arran	Large mesh
16. ii.	1	16	—	—	Bute Channel	Large mesh
				Population b		
1952						
4. vii.	3	36·0	33–39	—	Bute Channel	Small mesh
4. xii.	1	39	—	—	Bute Channel	Small mesh
18. xii.	13	24·9	22–28	1·90	Tarbert	Large mesh
19. xii.	♀ 2	36·5	30–43	—	Strachur to	Large mesh
19. xii.	♂ 1	31	—	—	Inveraray	Large mesh
1953						
15. i.	2	37·5	35–40	—	Bute Channel	Small mesh
16. i.	♀ 2	33·5	33–34	—	Mountstuart	Large mesh
16. i.	♂ 2	34·0	33–35	—	Mountstuart	Large mesh
20. i.	1	36	—	—	S.E. of Pladda	Large mesh
21. i.	♂ 2	35·0	—	—	S.W. of Sanda	Large mesh
21. i.	♀ 2	33·5	33–34	—	E. of Ailsa Craig	Large mesh
5. ii.	2	38	—	—	Mountstuart	Large mesh
11. ii.	♀ 2	37·5	36–39	—	E. side of Arran	Large mesh
16. ii.	♀ 3	36·3	34–38	—	Bute Channel	Large mesh
18. ii.	♀ 33	32·5	28–39	2·59	Off Ardrossan	Large mesh
18. ii.	♂ 23	34·2	29–40	2·56	Off Ardrossan	Large mesh
				Population c		
1951						
13. xii.	1	34	—	—	Mountstuart	Large mesh
1952						
14. iii.	4	41·0	40–42	—	Mountstuart	Large mesh
18. xii.	♀ 3	56·3	51–60	—	Tarbert	Large mesh
	♂ 4	50·0	45–53	—	Tarbert	Large mesh
19. xii.	♀ 15	48·3	45–53	2·19	Strachur to	Large mesh
	♂ 11	47·9	39–54	4·16	Inveraray	Large mesh
1953						
15. i.	1	52	—	—	Bute Channel	Small mesh
16. i.	1	49	—	—	Mountstuart	Large mesh
10. ii.	♀ 1	56	—	—	Holy Isle	Large mesh
13. ii.	♀ 4	51·0	47–55	—	Tarbert	Large mesh
	♂ 3	50·7	50–51	—	Tarbert	Large mesh
	8	55·1	51–59	3·36	Tarbert	Large mesh
				Population d		
1949						
14. x.	137	8·1	6–10	0·99	Bute Channel	Small mesh
17. x.	160	7·6	5–10	1·01	Bute Channel	Small mesh
26. x.	85	7·9	6–10	1·05	Loch Fyne	Small mesh
29. x.	38	9·5	6–13	1·27	Bute Channel	Small mesh
15. xi.	43	11·7	9–14	1·21	Bute Channel	Small mesh
22. xi.	17	12·2	10–15	1·52	Wemyss Bay	Small mesh
	125	12·1	8–18	1·70	Bute Channel	Small mesh
28. xi.	28	12·2	6–16	2·33	Bute Channel	Small mesh
1950						
18. i.	3	15·7	14–19	—	Bute Channel	Large mesh
19. i.	3	16·0	15–17	—	Bute Channel	Large mesh

TABLE I (*cont.*)

Date	No. in sample	Mean length	Length range	Standard deviation	Position of haul	Gear
			Population d (cont.)			
1950						
25. i.	1	15	—	—	Mountstuart	Large mesh
27. i.	5	16·8	14–19	—	Mountstuart	Large mesh
7. ii.	9	17·4	14–22	2·88	Mountstuart	Large mesh
10. ii.	5	20·0	19–21	—	Mountstuart	Large mesh
15. ii.	1	22	—	—	Mountstuart	Large mesh
17. ii.	8	15·9	12–20	2·70	Loch Fyne	Large mesh
20. ii.	6	18·0	16–21	1·79	Bute Channel	Large mesh
20. ii.	4	18·0	16–19	—	Mountstuart	Large mesh
27. ii.	1	18	—	—	Mountstuart	Large mesh
9. iii.	17	16·5	13–21	2·24	Kilbrennan Sound	Large mesh
21. iii.	13	17·5	15–19	1·39	Ailsa Craig	Large mesh
28. iii.	46	19·1	15–25	2·20	Inchmarnock Water	Large mesh
31. v.	3	20·0	19–21	—	Mountstuart	Large mesh
15. ix.	32	27·6	18–34	3·42	Mountstuart	Small mesh
15. ix.	37	27·9	21–34	2·93	Mountstuart	Large mesh
20. xi.	23	34·8	27–41	3·43	Mountstuart	Large mesh
1951						
15. i.	3	39·7	37–42	—	Mountstuart	Large mesh
7. ii.	6	34·0	25–42	5·83	Knock Castle	Large mesh
12. ii.	99	36·6	30–44	3·09	Mountstuart	Large mesh
21. ii.	41	37·2	27–44	3·79	Mountstuart	Large mesh
18. iv.	24	39·1	28–45	3·51	Mountstuart	Large mesh
23. v.	26	38·2	33–48	3·52	Mountstuart	Large mesh
23. v.	10	39·1	31–45	4·48	Knock Castle	Large mesh
11. vi.	29	40·4	33–47	3·50	Mountstuart	Large mesh
28. vi.	7	40·1	36–44	2·91	Mountstuart	Large mesh
28. vi.	19	41·0	36–48	1·17	Bute Channel	Large mesh
10. vii.	17	40·5	35–47	3·47	Mountstuart	Large mesh
14. viii.	16	41·3	34–47	3·48	Mountstuart	Large mesh
28. viii.	2	40·5	35–46	—	Mountstuart	Large mesh
11. ix.	4	46·8	45–50	—	Mountstuart	Large mesh
12. ix.	2	38·5	33–44	—	Mountstuart	Large mesh
15. x.	5	49·6	45–54	—	Bute Channel	Large mesh
21. xi.	5	47·0	42–50	—	Mountstuart	Large mesh
22. xi.	3	51·3	49–55	—	Bute Channel	Small mesh
1952						
8. i.	1	55	—	—	Mountstuart	Small mesh
12. ii.	9	55·2	52–60	3·03	Mountstuart	Small mesh
20. ii.	6	55·7	51–61	3·50	Bute Channel	Small mesh
14. iii.	8	53·0	49–59	2·93	Mountstuart	Large mesh
8. iv.	6	55·3	50–63	5·16	Mountstuart	Large mesh
14. iv.	6	53·8	52–59	2·71	Bute Channel	Small mesh
23. v.	1	50	—	—	Mountstuart	Large mesh
4. vii.	2	56·5	53–60	—	Bute Channel	Small mesh
11. xii.	♀ 14	68·9	63–75	3·24	Off Corrie, Arran	Large mesh
11. xii.	♂ 3	51·7	51–53	—	Off Corrie, Arran	Large mesh
18. xii.	♀ 16	68·1	61–75	3·98	Tarbert	Large mesh
18. xii.	♂ 18	63·3	55–72	4·30	Tarbert	Large mesh
19. xii.	♀ 59	62·6	53–72	4·43	Strachur to Inveraray	Large mesh
19. xii.	♂ 20	59·3	51–67	4·63		Large mesh
1953						
15. i.	1	66	—	—	Bute Channel	Small mesh
22. i.	♀ 3	68·3	67–69	—	Kilbrennan Sound	Large mesh
10. ii.	2	68·5	68–69	—	E. side of Arran	Large mesh
11. ii.	♀ 6	71·0	65–75	3·74	E. side of Arran	Large mesh
11. ii.	♂ 2	69·5	69–70	—	E. side of Arran	Large mesh
13. ii.	♀ 50	69·5	60–77	4·50	Tarbert	Large mesh
13. ii.	♂ 27	63·7	58–69	3·24	Tarbert	Large mesh
13. ii.	122	67·8	57–77	3·78	Tarbert	Large mesh
16. ii.	1	68	—	—	Bute Channel	Large mesh

Date	No. in sample	Mean length	Length range	Standard deviation	Position of haul	Gear
				Population e		
1949						
6. vii.	6	18·3	14–22	3·01	Inchmarnock Water	Large mesh
4. x.	4	31·5	30–33	—	Bute Channel	Large mesh
22. xi.	1	34	—	—	Wemyss Bay	Small mesh
2. xii.	4	30·3	26–33	—	Off Ardrossan	Large mesh
1950						
25. i.	14	38·6	33–45	3·76	Mountstuart	Large mesh
27. i.	53	37·6	31–47	3·95	Mountstuart	Large mesh
7. ii.	19	40·4	32–46	4·00	Mountstuart	Large mesh
10. ii.	58	38·2	27–51	4·65	Mountstuart	Large mesh
15. ii.	56	39·7	33–47	3·34	Mountstuart	Large mesh
17. ii.	12	44·4	39–50	3·73	Loch Fyne	Large mesh
20. ii.	6	41·0	36–50	4·82	Bute Channel	Large mesh
20. ii.	46	38·6	28–48	4·48	Mountstuart	Large mesh
27. ii.	42	39·0	31–46	3·26	Mountstuart	Large mesh
21. iii.	29	36·7	30–47	3·91	Ailsa Craig	Large mesh
31. v.	14	40·9	31–46	4·15	Mountstuart	Large mesh
15. ix.	7	46·6	41–52	4·35	Mountstuart	Small mesh
15. ix.	11	46·5	38–52	4·52	Mountstuart	Large mesh
20. xi.	4	50·5	47–55	—	Mountstuart	Large mesh
1951						
15. i.	2	51·5	47–56	—	Mountstuart	Large mesh
12. ii.	3	52·7	49–60	—	Mountstuart	Large mesh
21. ii.	6	51·5	47–55	2·81	Mountstuart	Large mesh
11. vi.	1	61	—	—	Mountstuart	Large mesh
10. vii.	2	61·0	60–62	—	Mountstuart	Large mesh
15. x.	1	61	—	—	Bute Channel	Large mesh
11. xii.	1	62	—	—	Fairlie Channel	Small mesh
1952						
11. xii.	♀ 3	83·3	83–84	—	Off Sannox, Arran	Large mesh
11. xii.	♂ 3	70·3	68–72	—	Off Sannox, Arran	Large mesh
18. xii.	♀ 2	84·0	82–86	—	Tarbert	Large mesh
18. xii.	♂ 3	75·0	73–78	—	Tarbert	Large mesh
19. xii.	♀ 5	75·8	71–80	—	Strachur to	Large mesh
19. xii.	♂ 1	73	—	—	Inveraray	Large mesh
1953						
22. i.	♀ 2	76·0	75–77	—	Kilbrennan Sound	Large mesh
10. ii.	♂ 1	82	—	—	Off Holy Isle	Large mesh
11. ii.	♀ 2	84·5	82–87	—	Off Sannox, Arran	Large mesh
13. ii.	♀ 7	80·0	74–86	4·04	Tarbert	Large mesh
13. ii.	♂ 7	73·3	71–77	1·98	Tarbert	Large mesh
13. ii.	34	77·9	72–88	4·28	Tarbert	Large mesh
16. ii.	♀ 1	77	—	—	Mountstuart	Large mesh
				Population f		
1949						
6. vii.	2	41·5	38–45	—	Inchmarnock Water	Large mesh
6. vii.	2	62·5	62–63	—	Inchmarnock Water	Large mesh
1950						
19. i.	1	90	—	—	Bute Channel	Large mesh
17. ii.	6	60·8	56–65	3·31	Loch Fyne	Large mesh
17. ii.	2	71	—	—	Loch Fyne	Large mesh
17. ii.	10	80·0	75–87	3·94	Loch Fyne	Large mesh
20. ii.	1	58	—	—	Mountstuart	Large mesh
27. ii.	1	54	—	—	Mountstuart	Large mesh
1953						
22. i.	4	103·0	93–110	—	Kilbrennan Sound	Large mesh
11. ii.	1	92	—	—	Off Sannox, Arran	Large mesh

which are believed to have been hatched during the summer of 1949, were first caught during October of that year, when they had reached a mean length of 8 cm., and ranged from 5 to 10 cm. The catches obtained at this time are of very great interest as hake of this size had not been caught before in any quantity.

Post-larval hake of under 2 cm. are well known; they have been described by Schmidt (1907) and reported from the Plymouth area (Clark, 1920; Russell, 1930–7; Corbin, 1948), and from S.W. Ireland (Schmidt, 1909; Hickling, 1933). However, very little indeed is known about the adolescent post-larval stage; Hickling (1933) only records nineteen fish of 10 cm. and less, which he himself caught in 1932. During the autumn of 1949, 452 young hake of this size were caught in the Clyde area. The good catches of this year class ended in January 1950 owing to the loss of the small-mesh trawl, although some of these fish have been caught almost every month up to the end of the sampling period.

Population *e* consists of 473 fish which hatched in 1948, growing to 18 cm. by June 1949 and 78 cm. by February 1953.

Population *f* consists of all fish longer than those that fit into population *e*. There is no evidence that they are a homogeneous collection, and they have been listed together only for convenience; they do not contribute materially to the results.

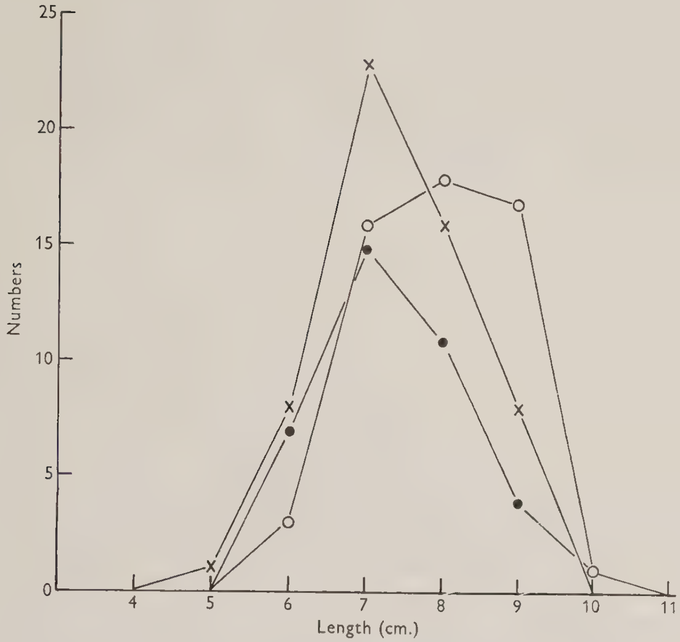
Reliability of the Samples

If fish measurements are to be used for the determination of growth rate it is essential that the catches should give an adequate indication of the size-frequency distributions of the populations. To test the reliability of the samples three series of hauls were considered and the means within each series compared; in no comparison was a significant difference found.

Series I is of three hauls taken with the small-mesh trawl in Bute Channel on 17 October 1949. The data are given in Table II, and Text-fig. 1; there is no significant evidence that it was not the same population that was sampled in each haul.

Series II compares two hauls taken with the large-mesh trawl off Inveraray on 19 December 1952. The data are given in Table III and Text-fig. 2; again the same population has been sampled in both hauls.

Series III, caught on 15 September 1950, and given in Table IV and Text-fig. 3, shows that the two kinds of gear used may be relied upon to give comparable samples from the same population, provided the fish are large enough not to be selected by the size of mesh. The large-mesh trawl caught thirty-seven fish with a mean length of 27·9 cm., while the small-mesh gear obtained thirty-two with a mean length of 27·6 cm.



Text-fig. 1. Length-frequency distributions of hake taken in series I. ×, haul I; o, haul II; ●, haul III.

TABLE II. SERIES I SIZE-FREQUENCY DISTRIBUTION, ILLUSTRATED IN TEXT-FIG. 1

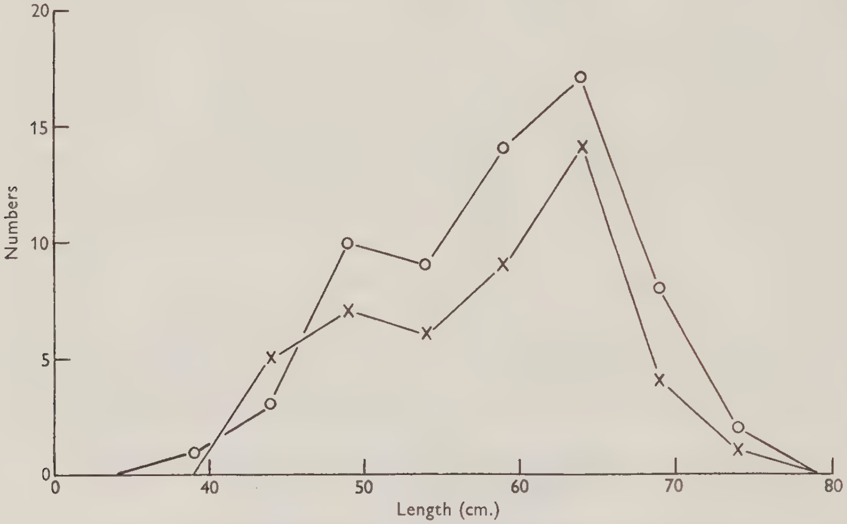
Length (cm.)	Number of hake Haul I	 Haul II	 Haul III
5	1	—	—
6	8	3	7
7	23	16	15
8	16	18	11
9	8	17	4
10	—	1	1
Total	56	55	38
Mean	7·4	7·9	7·4

GROWTH RATE

The growth rate of the Clyde hake has been determined by Petersen's method, based on size-frequency distributions, and confirmed by examination of the otoliths of the fish caught during December 1952 and January and February 1953.

From Size-Frequency Distributions

Petersen's method of age determination and deduction of the growth rate depend upon two conditions: first, that the fish spawn during a limited period and at approximately the same date each year; and secondly, that they grow at about the same rate. If these conditions are fulfilled and a sample of the population is measured, the age-groups present can be found by inspection of the size frequencies. If the young of one particular year are especially numerous, the growth of that year-class can be followed over a period of time from size-frequency graphs of successive samples.

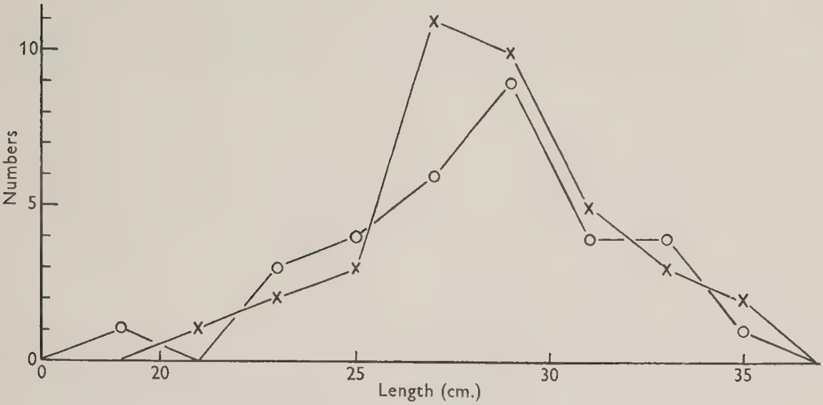


Text-fig. 2. Length-frequency distributions of hake taken in series II. ×, haul I; o, haul II.

TABLE III. SERIES II SIZE-FREQUENCY DISTRIBUTION, ILLUSTRATED IN TEXT-FIG. 2

Size-groups (cm.)	Number of hake	
	Haul I	Haul II
37–41	—	1
42–46	5	3
47–51	7	10
52–56	6	9
57–61	9	14
62–66	14	17
67–71	4	8
72–76	1	2
Total	46	64
Mean	58·1	58·9

The data given for each population in Table I have been summed for each month and are plotted in the graph in Text-fig. 4. It can be seen that there was never, during any month, an overlap of the ranges of any two populations except during December 1952 and January and February 1953, when a special effort had been made to obtain as many hake as possible, some 580 being caught. Because these monthly samples are considerably larger than the others, their ranges do overlap and have therefore not been added to Text-fig. 4; for these months, only the mean lengths and standard deviations



Text-fig. 3. Length-frequency distributions of hake taken in series III. ×, Large mesh; o, Small mesh.

TABLE IV. SERIES III SIZE-FREQUENCY DISTRIBUTION, ILLUSTRATED IN TEXT-FIG. 3

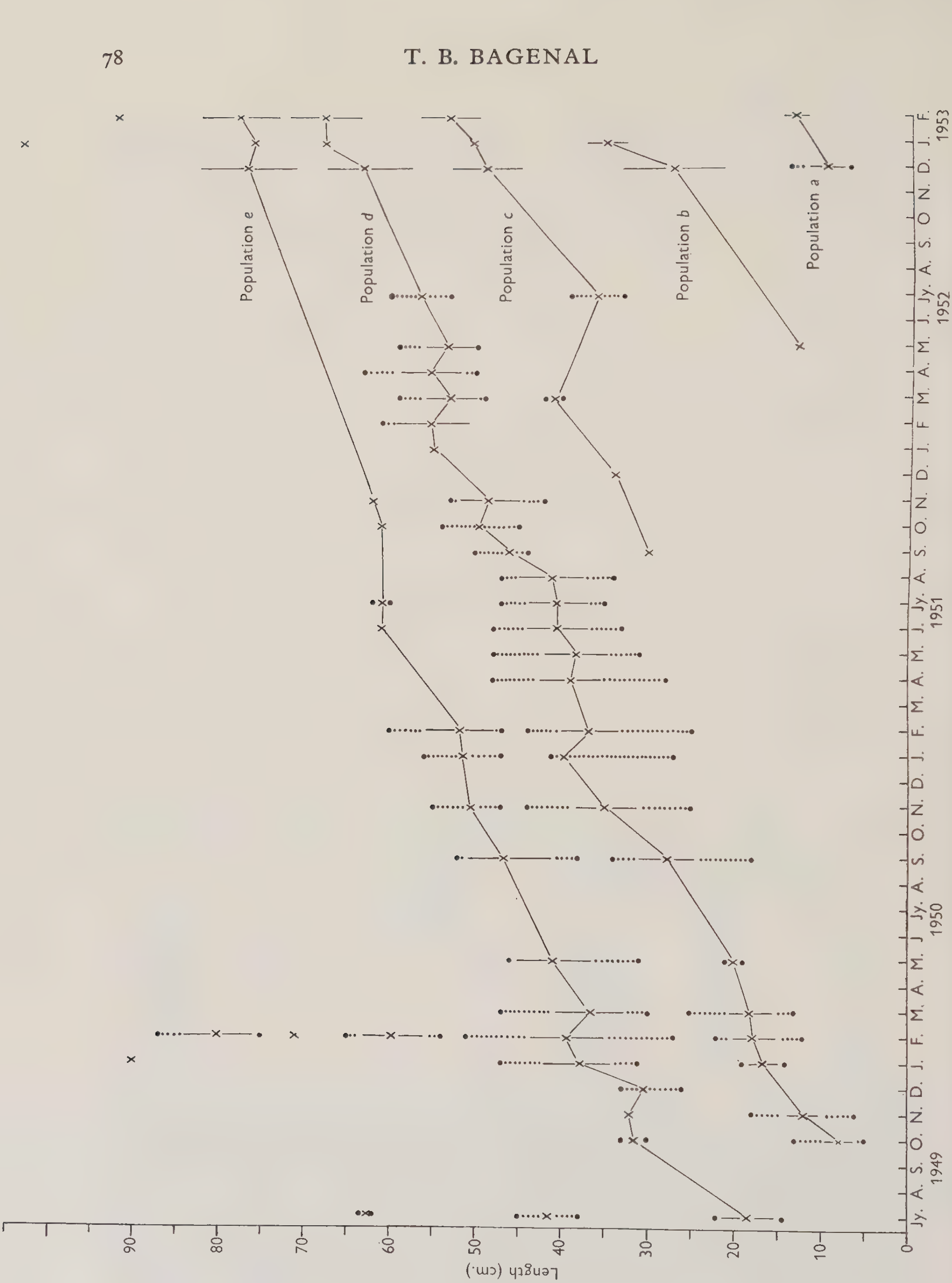
Size groups (cm.)	Large mesh	Small mesh	Size groups (cm.)	Large mesh	Small mesh
18–19	—	1	30–31	5	4
20–21	1	—	32–33	3	4
22–23	2	3	34–35	2	1
24–25	3	4	Total	37	32
26–27	11	6	Mean	27·9	27·6
28–29	10	9			

TABLE V. MEAN LENGTHS OF EACH POPULATION AT VARIOUS AGES, OBTAINED BY INSPECTION OF TEXT-FIG. 4, TOGETHER WITH THE MEAN LENGTHS DURING FEBRUARY 1953

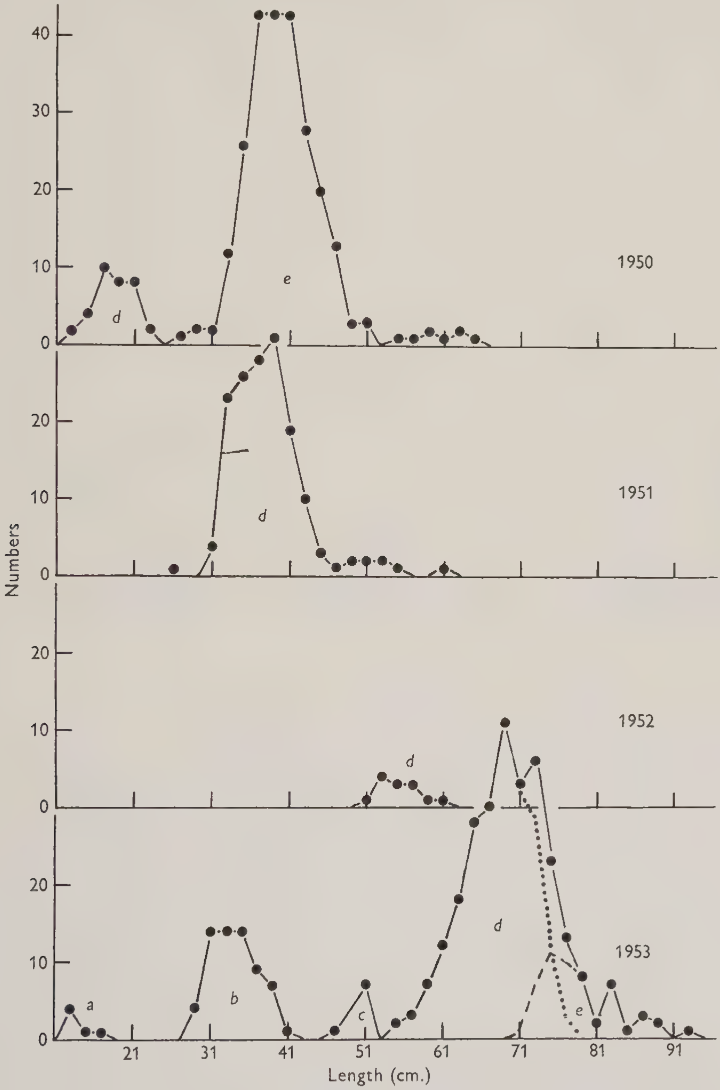
		Mean length (cm.) at				
Population	Hatched	1 year	2 years	3 years	4 years	Length, Feb. 1953
e	1948	18·3	44·0	61	*c.* 70	77·8
d	1949	24·0	40·5	56·5	—	67·8
c	1950	*c.* 28	*c.* 45	—	—	53·3
b	1951	*c.* 18–20	—	—	—	33·6
a	1952	—	—	—	—	13·5
Mean	—	22·3	43·2	58·7	70	—

are given. The graph shows that the populations are distinct and homogeneous groups, and the growth rate of the hake may be deduced from the growth rate of each population, and this information, extracted from Text-fig. 4, is given in Table V.

The data for each February of 1950, 1951, 1952 and 1953 are plotted in Text-fig. 5, which confirms that the populations are distinct, except in February 1953 when population *e* (marked with a dashed line) was masked



Text-fig. 4. Graph showing the mean (×), ± one standard deviation (——), and the range (......) of each hake population by population *d*, but this was discovered only from the results of otolith readings. With large samples of relatively large fish, it is considerably more



Text-fig. 5. Length-frequency diagram of hake caught each February of 1950, 1951, 1952, and 1953. The letters *a*–*e* refer to the populations shown in Table I.

difficult to pick out the various age-classes, as, with increasing age, there is a greater possibility of overlap, and with increasing numbers, there is a greater possibility of catching the extremes of range.

From direct Otolith Readings

In December 1952 it was decided that corroborative evidence was needed to confirm the growth rate suggested by the size frequencies. Hake were obtained from as widely scattered localities in the Clyde area as possible, measured, sexed and the otoliths extracted. Otoliths were chosen for age determination because they have been found to be the most satisfactory by previous workers (see Birtwistle & Lewis, 1925; Hickling, 1933). The otoliths were examined as soon as possible after they had been extracted since it was found that after drying out it was considerably more difficult to see any concentric bands. The otoliths were placed in water in a black dish illuminated from above by a strong spot-light, and examined with the naked eye. It could then be seen that the otoliths exhibited opaque zones with intervening translucent bands (Pl. I); the centre was almost always opaque. On the assumption that one of each of these structures is formed annually, the translucent bands were counted, and the number found was taken to be the age of the fish in years. This assumption is reasonable since fish of equal length were nearly always found to be of equal age, and the age structure of the samples was found to agree with that obtained by other methods.

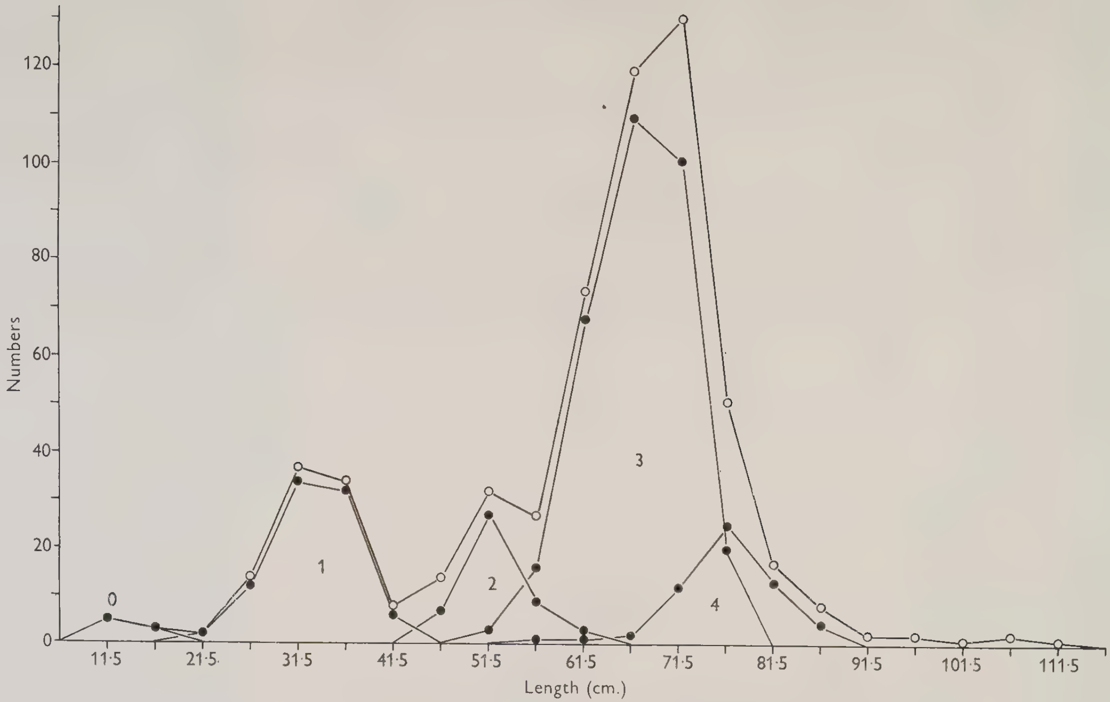
Altogether otoliths were taken from 585 fish and the results of their examination are given in Table VI, and in Text-fig. 6, where the size frequencies of the fish are shown. It can be seen that there is close agreement between the distribution of the age-groups and the modes of the total size-frequency graph.

From Back-calculation of Otolith Readings

If there is a simple relationship between the length of the otolith and the length of the fish, it should be possible to calculate the size of the fish when each of the translucent bands was laid down; that is once a year throughout the life of the fish. Therefore all the overall lengths of the otoliths that were extracted undamaged were measured to the nearest millimetre; the results are given in Table VII, together with the range and mean of the corresponding fish length, and in Text-fig. 7, where it can be seen that the points lie approximately on a straight line. A selection of the otoliths were re-examined and the lengths at each translucent band measured. It was found that the most satisfactory method was to store the otoliths in alcohol and examine them in creosote in a black dish illuminated from above. Using this method the otoliths were photographed and the translucent bands measured from the image of the negative placed in an enlarger.

It was found sometimes that the rings, though easily counted, were not necessarily clear on the long axis of the otolith; for this reason readings were not obtained from all the otoliths examined.

From these measurements the sizes of the fish during the formation of each ring were calculated, with the results given in Table VIII. In considering them



Text-fig. 6. Length-frequency diagrams of the various hake age-groups, 0–4 (●), as indicated by their otoliths, and of all the hake examined (o).

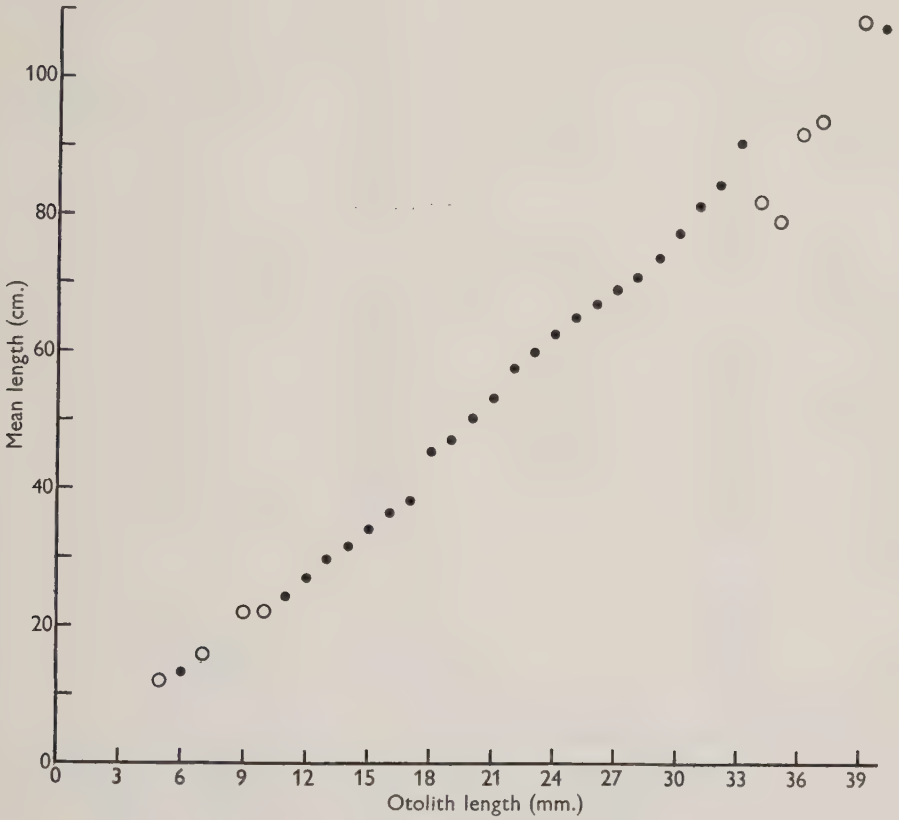
it must be remembered, first, that for each size of otolith there is often a considerable range in size of fish (see Table VII); and secondly that the otolith length is about 0·04 of the length of the fish, so that any inaccuracy in the otolith measurement (even taken from an enlarged picture) leads to a considerably greater inaccuracy in the calculated fish length.

TABLE VI. SIZE-FREQUENCY DISTRIBUTIONS OF HAKE AGE-CLASSES, DETERMINED BY OTOLITH READINGS, TOGETHER WITH TOTAL SIZE-FREQUENCY AND DOUBTFUL READINGS

Size-groups (cm.)	Total frequency	Age-group frequencies					Illegible and uncertain
		0	1	2	3	4	
9– 13	5	5	—	—	—	—	—
14– 18	3	3	—	—	—	—	—
19– 23	2	—	2	—	—	—	—
24– 28	14	—	12	—	—	—	2
29– 33	37	—	34	—	—	—	3
34– 38	34	—	32	—	—	—	2
39– 43	8	—	6	—	—	—	2
44– 48	14	—	—	7	—	—	7
49– 53	32	—	—	27	3	—	2
54– 58	27	—	—	9	16	1	1
59– 63	74	—	—	3	68	1	2
64– 68	120	—	—	—	110	2	8
69– 73	131	—	—	—	101	12	18
74– 78	51	—	—	—	20	25	6
79– 83	17	—	—	—	—	13	4
84– 88	8	—	—	—	—	4	4
89– 93	2	—	—	—	—	—	2
94– 98	2	—	—	—	—	—	2
99–103	1	—	—	—	—	—	1
104–108	2	—	—	—	—	—	2
109–113	1	—	—	—	—	—	1
Total	585	8	86	46	318	58	69
Mean	—	13·5	32·7	51·8	66·5	76·0	—

Review of all Evidence

The data on the growth rate of the Clyde hake have been obtained from size-frequency distributions and from otolith readings; and each method has given estimates of the lengths, both at the end of each of the first 4 years of life, and at approximately the middle of each of the first 5 years. Data summarizing the previous sections are given in Table IX, and in Text-fig. 8. It will be noticed that all these methods give very consistent results. The slightly smaller estimates obtained from the otoliths are due to several factors: first, the mean sizes of the age-groups caught during February 1953 (*a* in Table IX) apply to the last of the 3 months during which otoliths were extracted (*c*); and secondly, the estimates for each population given in Table V (*b* in Table IX) were obtained by inspection from Text-fig. 4, while the possibilities of errors in otolith back-calculation (*d*) have already been stressed; and thirdly, it is not certain what date approximates to the mid-point of each growth-year for Clyde hake. The otoliths of only one hake, a male of 25 cm. caught on 21 January 1953, have suggested a growth rate which does not agree with that outlined in this paper. The otolith showed three very clear translucent bands which were quite unlike subsidiary rings. Unfortunately, the fish was not kept after the otolith was removed so it was impossible to determine what had caused the slow growth.



Text-fig. 7. The relationship between the length of hake and the length of their otoliths. o, only one otolith examined.

Comparison with Previous Studies

When le Danois (1920) published his summary of the existing knowledge of the hake, it was clear that at that time very little was known about the growth rate. The first work on this was by Belloc (1923), who examined scales from 264 fish obtained from three localities: Morocco, the Bay of Biscay and south of Ireland, and he suggested that there are racial differences in the growth rate in the three regions. Later, he republished this data adding some observations

TABLE VII. OTOLITH SIZE-FREQUENCY WITH ASSOCIATED FISH LENGTHS

Otolith length (mm.)	Frequency	Range of fish length (cm.)	Mean length of fish (cm.)
5	1	12	12
6	8	13–14	13·2
7	1	16	16
8	—	—	—
9	1	22	22
10	1	22	22
11	8	23– 27	24·4
12	13	24– 31	27·0
13	14	28– 31	29·8
14	38	28– 36	31·8
15	44	30– 38	34·3
16	14	35– 40	36·7
17	10	35– 43	38·4
18	4	45– 46	45·5
19	13	44– 51	47·2
20	25	47– 57	50·6
21	38	49– 67	53·4
22	22	50– 67	58·0
23	51	53– 71	60·3
24	75	55– 74	63·1
25	93	55– 76	65·8
26	126	52– 82	67·3
27	143	61– 82	70·0
28	83	65– 80	71·6
29	49	67– 98	74·5
30	23	71– 98	78·1
31	10	77– 88	82·1
32	7	80– 92	85·6
33	3	87–101	91·7
34	1	83	83
35	1	80	80
36	1	93	93
37	1	95	95
38	—	—	—
39	1	110	110
40	2	108–110	109

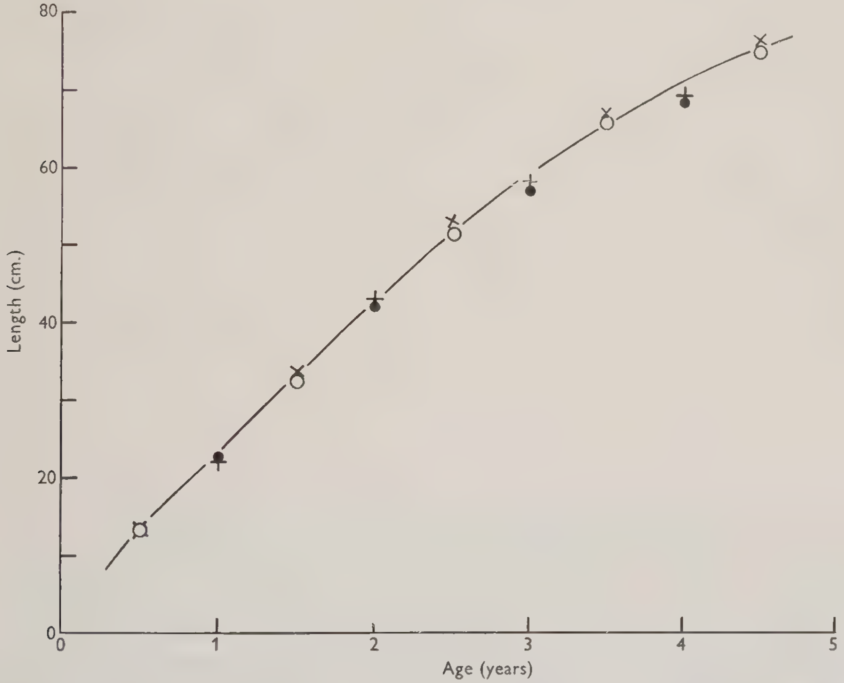
TABLE VIII. THE MEAN LENGTHS (CM.) OF FISH OF VARIOUS AGES AS DETERMINED BY OTOLITH BACK-CALCULATION

		Mean lengths (cm.) of hake calculated from otoliths showing							
		Two translucent bands		Three translucent bands		Four translucent bands			
Age (years)		No.	Length	No.	Length	No.	Length	Mean length (cm.)	Grand mean length (cm.)
1	♂	5	22·22	23	22·29	3	22·77	22·14	22·59
	♀	6	22·16	44	22·32	7	24·64	23·04	
2	♂	4	39·04	23	43·49	2	43·09	41·87	42·48
	♀	6	38·08	46	43·44	7	47·77	43·10	
3	♂	—	—	7	54·42	3	58·11	56·26	57·52
	♀	—	—	22	58·22	6	59·34	58·78	
4	♂	—	—	—	—	1	65·94	65·94	69·26
	♀	—	—	—	—	3	72·58	72·58	

on a slow-growing stock found in the Mediterranean (Belloc, 1929). His results are given in Table X and compared with the Clyde results in Text-fig. 9. It can be seen that even the fastest-growing race, those from the Morocco Coast, only reach a size of about 79 cm., 9 years after hatching, whereas the Clyde hake reach a comparable size during their fifth year.

TABLE IX. SUMMARY OF ALL EVIDENCE OF MEAN FISH LENGTHS AT DIFFERENT AGES; ILLUSTRATED IN TEXT-FIG. 8

Age (years) ...	0+	1	1+	2	2+	3	3+	4	4+
From length frequencies									
(*a*) Mean length, Feb. 1953	13·5	—	33·6	—	53·3	—	67·8	—	77·8
(*b*) Mean of all populations (Table V)	—	22·3	—	43·2	—	58·7	—	70·0	—
From otoliths									
(*c*) Direct reading (Table VI)	13·5	—	32·7	—	51·8	—	66·5	—	76·0
(*d*) Back-calculations (Table VIII)	—	22·6	—	42·5	—	57·5	—	69·3	—
Mean	13·5	22·4	33·1	42·8	52·5	58·1	67·1	69·6	76·9



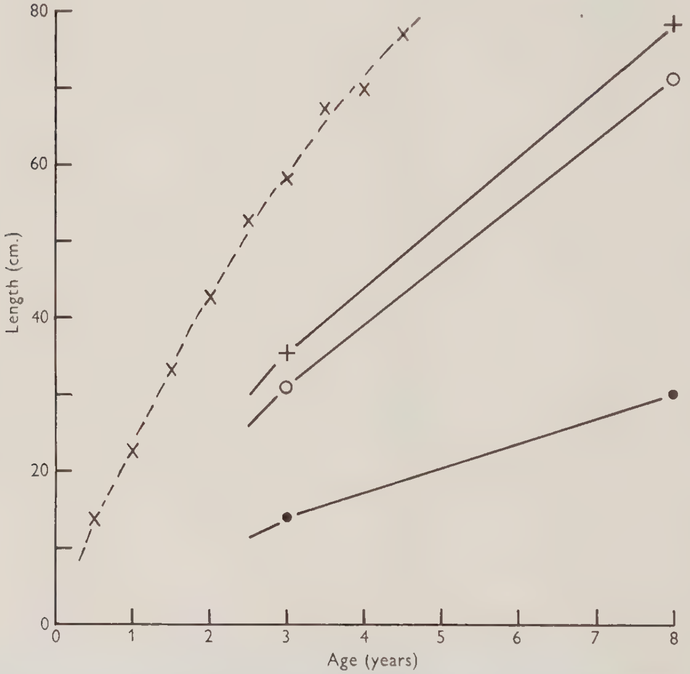
Text-fig. 8. Summary of growth of hake from all evidence. +, from length-frequency measurements, mean of all populations; ×, from length frequencies, mean lengths in February 1953; o, from direct otolith readings, mean lengths of each age-group; ●, from back-calculation of otoliths, mean of all populations at each age.

The first British work published was that of Birtwistle & Lewis (1925), who studied the hake in the Irish Sea and to the north-west and south-west of

Ireland. These authors used scales and otoliths for age determination; although they found scales very difficult to read, they were forced to use them for all marketable fish. Their results are given in Table XI and compared graphically with the present observation in Text-fig. 10. The growth rates of the fish studied by Birtwistle & Lewis are all slower than that described in the present paper, although the findings for the Irish Sea are quite comparable to those of the Clyde.

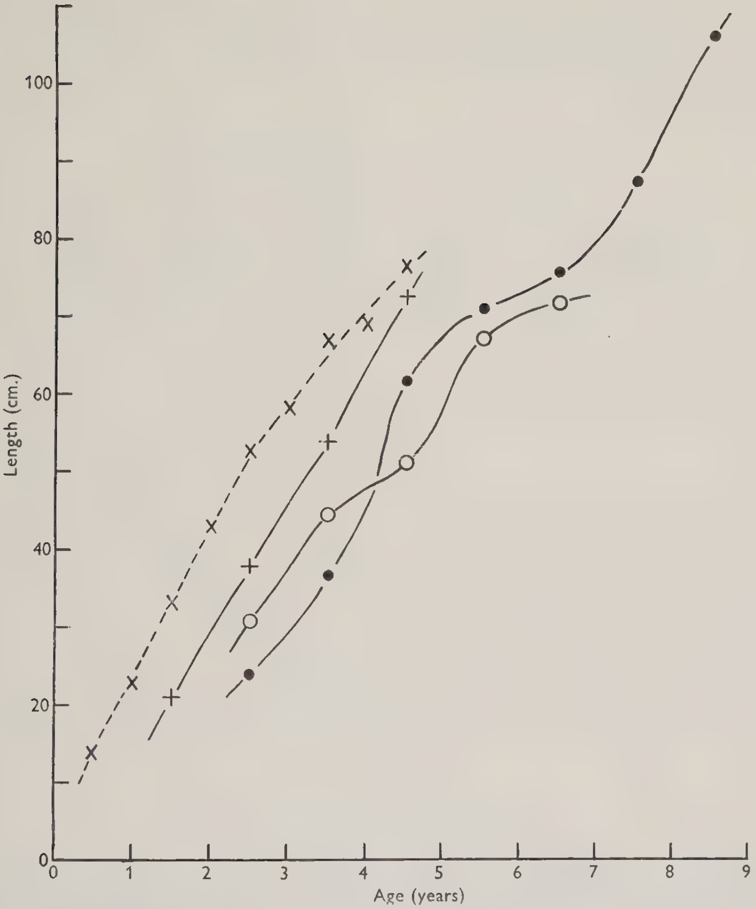
TABLE X. MEAN LENGTHS (CM.) OF HAKE OF VARIOUS AGES FROM FOUR REGIONS, ACCORDING TO BELLOC

Years after hatching	Morocco	Bay of Biscay	Ireland	Mediterranean
3	35	31	—	14
8	78	71	65	30
13	87	82	75	36



Text-fig. 9. Comparison of hake growth rate given by Belloc (+—+, Morocco; o—o, Bay of Biscay; ●—●, Mediterranean) with that given in Text-fig. 8 for the Clyde (× - - - ×).

In 1933 Hickling published the fourth part of his 'Natural History of the Hake', in which he considers age determination and growth rate. His results are based on the measurements of 54,560 hake, of which he examined the otoliths of 25,930; as he aptly remarks 'We therefore have plentiful material on which to base our results'. These results are shown in Table XII (taken



Text-fig. 10. Comparison of hake growth rate given by Birtwistle & Lewis (+—+, Irish Sea; ●—●, S.W. Ireland; o—o, N.W. Ireland) with that given in Text-fig. 8 for the Clyde (×---×).

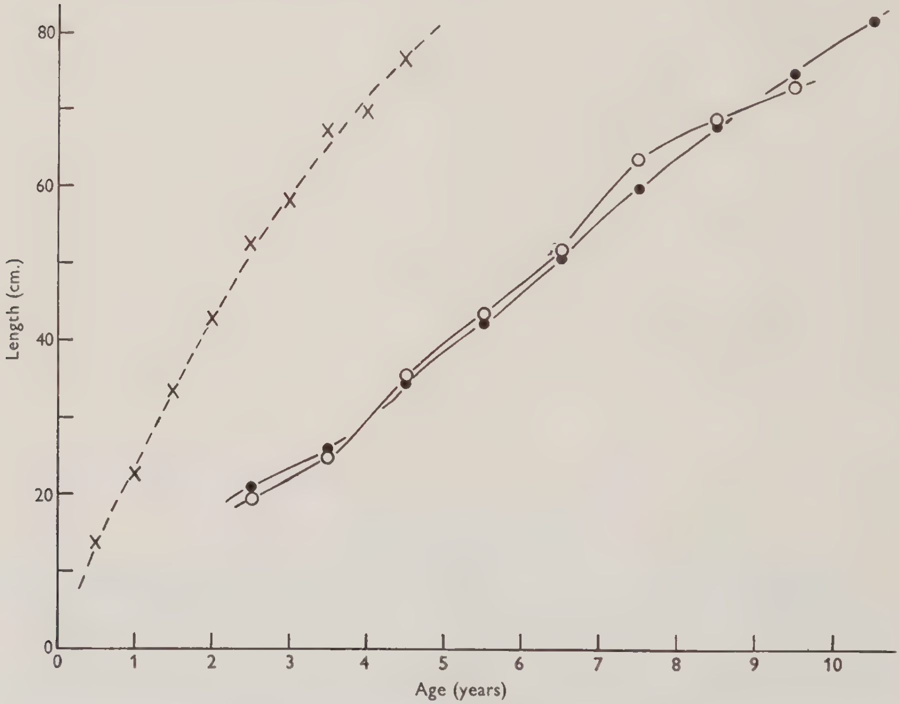
Table XI. Mean Lengths (cm.) of Hake of Various Ages from Three Regions, According to Birtwistle & Lewis

Age	Irish Sea	S.W. Ireland	N.W. Ireland
1	20·3	—	—
2	37·5	23·7	30·7
3	54·0	36·8	44·3
4	72·5	61·9	51·7
5	—	71·8	67·5
6	—	76·3	72·5
7	—	88·3	—
8	—	107·5	—

from his table IX*c*). Text-fig. 11 compares graphically the results of Hickling for the south and west of Ireland and also the Clyde with those of the present work, and it will be seen that there is a very marked discrepancy between the results of the two investigations.

TABLE XII. MEAN LENGTHS (CM.) OF HAKE OF VARIOUS AGES FROM TWO REGIONS, ACCORDING TO HICKLING

Age-groups ...	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XII+
S. and W. of Ireland, 1928–32	20·9	25·6	34·6	42·1	50·9	59·8	67·9	74·1	81·2	86·2	82·9	97·0
W. of Scotland, 1932	19·6	25·4	35·1	43·2	51·4	63·4	68·0	72·9	—	—	—	—



Text-fig. 11. Comparison of hake growth rate given by Hickling (●—●, south and west of Ireland; o—o, Clyde), with that given in Text-fig. 8 for the Clyde (×- - -×).

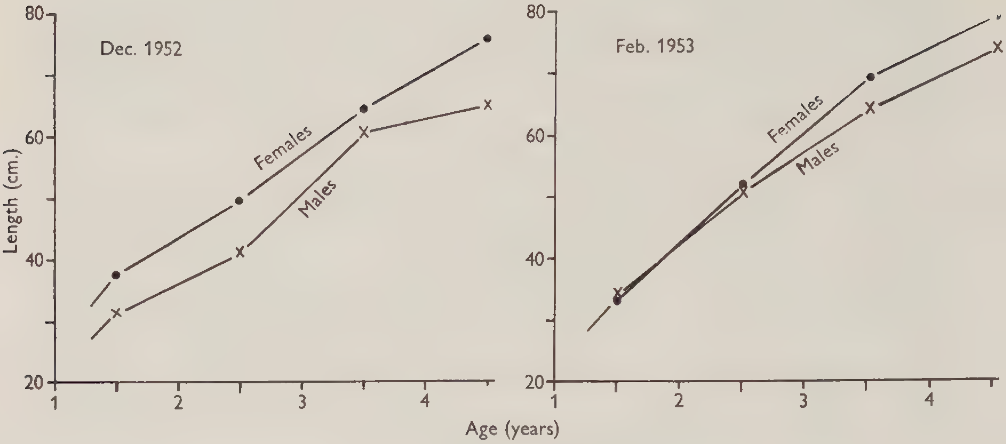
Hickling (1933) explained the difference between his results and those of Birtwistle & Lewis by suggesting that the latter authors had misread the scales and halved the age of each fish, because they suggested that a check ring appears on the scale when the growth slows down in the winter, and another faint ring is formed when the summer growth recommences, so that two rings correspond to 1 year's growth. Hickling pointed out that their results agree with his if one allots one zone to each year of life of the fish, but he failed to note that Birtwistle & Lewis did not suggest that the system of double-ringing applies to otoliths as well as to scales, and that their results for the small fish (obtained from otoliths) fit nicely to those for the larger fish obtained from scales.

Belloc (1929) maintained that he saw alternating clear and cloudy zones in the scales, and that one of each corresponds to a year's growth. Unfortunately he did not give any other supporting evidence for the growth rate he suggested; the numbers of fish caught in any month are not sufficient to provide any evidence from their size-frequency distribution. It is also unfortunate that Belloc did not state if there was a check ring, such as Birtwistle & Lewis saw, between his clear and cloudy zones. If there was not, and the ring was represented by the cloudy zone, it would be necessary to halve the age of his fish; this would make his results more comparable to those given in this paper, and to those reported by Birtwistle & Lewis.

Hickling's conclusions, on the other hand, are definitely based on sufficient material, and he provides adequate supporting evidence. Moreover, he illustrates his method of age determination by means of an excellent photograph of four otoliths (his figure 11), and there can be little doubt that his interpretations are correct. Nevertheless, the mean annual increase in length from all sources, is given as 8·7 cm., while elsewhere (footnote on p. 20) an account is given of the only successful hake-marking experiment; the fish grew 11·7 cm. in 8 months which would correspond to an annual increment of 17·5 cm. No explanation is given of this discrepancy.

Sexual Growth-Rate Differences

As reported by other workers (Belloc, 1929; Birtwistle & Lewis, 1925; Hickling, 1933), it has been found that the female hake grow faster than the males. The mean sizes of the age-groups of the two sexes during December 1952 and January and February 1953 are given in Table XIII, and for two of the months the data are shown graphically in Text-fig. 12. In Table XIV comparisons are made of the sizes of the two sexes caught in the same hauls on various dates, and it can be seen that the mean length of the females becomes very significantly larger during the third year. With the 2-year-old fish, although the females are larger, only in one sample is the difference possibly statistically significant. The 1-year-old fish are somewhat anomalous because in both samples the males are the larger. In the bigger haul taken on 18 February 1953, the larger size of the males is very definitely significant. In contrast, from the results of the back-calculations of the otoliths (Table VIII), it appears that it is more usual for both sexes to be of approximately the same size until they enter their third year of life when the large size of the females becomes more clearly recognizable and consistent.



Text-fig. 12. Comparison of growth rates of male and female hake.

TABLE XIII. MEAN LENGTHS (CM.) OF MALE AND FEMALE HAKE OF VARIOUS AGES DURING DECEMBER 1952 AND JANUARY AND FEBRUARY 1953

Age-group	...	1	2	3	4
December	♂	31·0	41·0	60·5	64·7
	♀	37·5	49·6	64·6	75·8
January	♂	35·7	—	—	—
	♀	33·5	50·5	67·7	76·0
February	♂	34·2	50·6	64·3	74·4
	♀	33·1	52·0	69·6	79·9

TABLE XIV. COMPARISON OF MEAN LENGTHS (CM.) OF MALE AND FEMALE HAKE CAUGHT IN THE SAME TRAWL HAUL

		Males		Females		
Date	Age	No.	Mean	No.	Mean	Difference between mean lengths
16. i. 53	1	2	34·0	2	33·5	—
18. ii. 53	1	23	34·2	33	32·5	Highly signif., $P<0·01$
18. xii. 52	2	4	50·0	3	56·3	—
19. xii. 52	2	11	47·9	15	48·3	Not signif., $P=0·4$
13. ii. 53	2	3	50·6	4	51·0	—
11. xii. 52	3	3	51·7	14	68·9	—
18. xii. 52	3	18	63·3	16	68·7	Highly signif., $P<0·01$
19. xii. 52	3	20	59·3	59	62·6	Highly signif., $P<0·01$
16. i. 53	3	2	69·5	6	71·0	—
13. ii. 53	3	27	63·7	50	69·5	Highly signif., $P<0·01$
11. xii. 52	4	3	70·5	3	83·3	—
18. xii. 52	4	3	75·0	2	84·0	—
19. xii. 52	4	1	73·0	5	75·8	—
13. ii. 53	4	7	73·3	7	80·0	Highly signif., $P<0·01$

Regional Size Differences in the Clyde Area

In all parts of the Clyde area hake of a given age have been found to have a similar mean size, though a few of the minor irregularities should be noted. The hake caught at the head of Loch Fyne between Strachur and Inveraray, on 19 December 1952, were consistently smaller than those of the same age caught just north of Tarbert the day before (Table XV). It is indeed very remarkable to find hake so plentiful (115 were caught in 1½ hr.) in such a land-locked position as Inveraray; it must be remembered that typically the species is found on the edge of the continental shelf.

TABLE XV. COMPARISON OF HAKE CAUGHT OFF TARBERT AND INVERARAY

Age ...	1	2		3		4	
Sex ...	♂+♀	♂	♀	♂	♀	♂	♀
Tarbert, 18. xii. 53	24·9	50·0	56·3	63·3	68·1	75·0	84·0
Inveraray, 19. xii. 53	37·5	47·9	48·7	62·6	59·3	73·0	75·8

TABLE XVI. LENGTH COMPARISON OF HAKE CAUGHT OFF MOUNTSTUART AND IN BUTE CHANNEL

	20. ii. 50		28. vi. 51	21 and 22. xi. 51
Population ...	*e*	*d*	*d*	*d*
Bute Channel	41·0	18·0	41·0	51·3 (Small Mesh)
Mountstuart	38·5	18·0	40·1	47·0 (Large Mesh)

Often the depth at which the fish were caught is responsible for differences in the sizes obtained. As a general rule the larger fish are found at the greater depth. This may be seen by comparing hauls from the Mountstuart ground (*c.* 50 m.) with some from the middle of Bute Channel (*c.* 80 m.) 1½ miles away (Table XVI). This depth effect is seen also by comparing the Mountstuart catch of 15 February 1950 (see Table I) when fish of 39·7 cm. mean size were caught, with the Loch Fyne catch 2 days later, at a depth of *c.* 200 m., when the mean size was 44·4 cm.

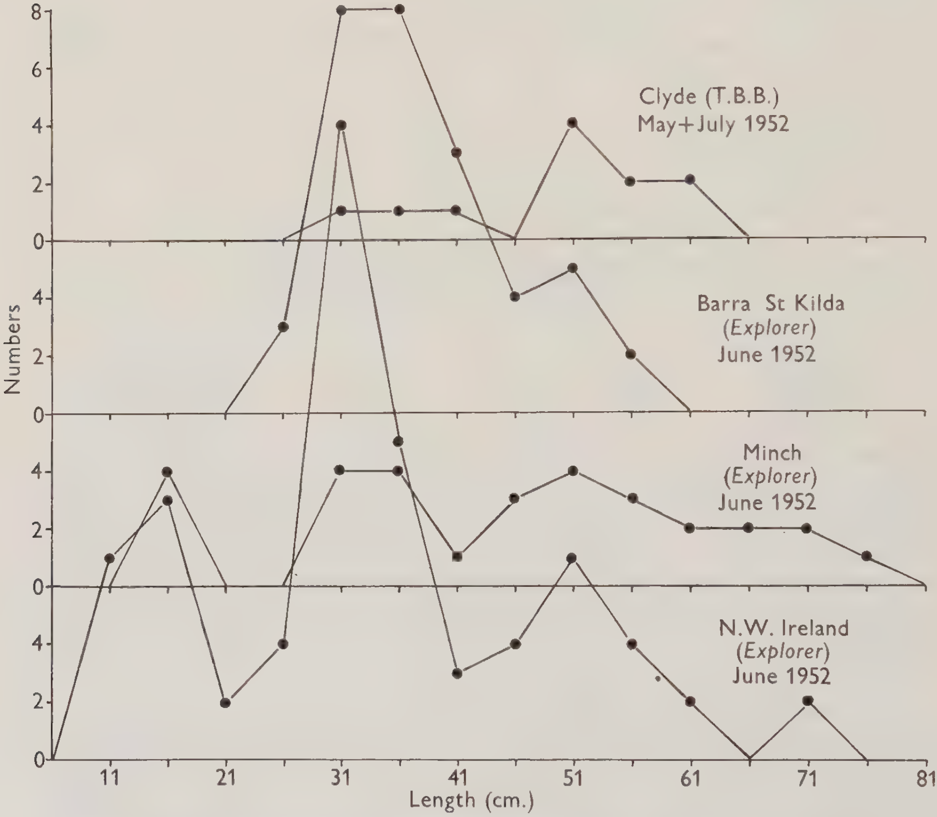
The Mountstuart and Knock Castle grounds were both fished on 23 May 1951, when hake of Population *d* were caught averaging 38·2 and 39·1 cm. respectively. The depth is approximately the same at both stations. However, the depth effect is most marked with the larger fish of 80 cm. and above. In only one instance, when one fish was obtained, have populations averaging 80 cm. and more been caught elsewhere than in the deep water of Loch Fyne and on both sides of Arran.

Growth Rate in other Scottish Areas

Through a kind invitation from the Marine Laboratory of the Scottish Home Department it was possible to join the F.R.S. *Explorer* on a west

Scotland cruise during June 1952, and thanks are due to Dr B. B. Rae for permission to use the hake length data that were obtained.

Hauls were taken at stations scattered round the north and west Scottish coasts, together with a few in the Moray Firth area and the Irish Sea. The hake lengths, measured to the nearest centimetre, have been grouped into 5 cm. classes and the length distributions summed for seven regions. These data are given in Table XVII, and Text-fig. 13, where Clyde figures for May-plus-July are also plotted for comparison.



Text-fig. 13. Length-frequency diagrams of hake from three of the Scottish areas (F.R.S. *Explorer*, June 1952), and from the Clyde during May and July 1952.

The size frequencies suggest that there are 5 year-classes present; the means of each have been calculated and are given in Table XVIII.

The yearly increments obtained from Tables XVIII and IX are given in Table XIX; the contrast between all these results and the mean increment of 8·7 cm./year suggested by Hickling (1933) is very marked.

TABLE XVII. LENGTH (CM.) FREQUENCY DISTRIBUTIONS OF HAKE FROM SEVEN SCOTTISH AREAS. F.R.S. *EXPLORER* DATA

Length (cm.)	North Sea	N. Scotland	Barra-St Kilda	Minch	N.W. Ireland	Clyde	Irish Sea
10–13	—	—	—	—	7	—	—
14–18	—	—	—	4	9	—	—
19–23	—	—	—	—	2	—	—
24–28	—	—	3	—	4	—	—
29–33	1	—	14	4	22	—	—
34–38	1	1	14	4	11	—	—
39–43	—	—	9	1	3	—	—
44–48	—	1	4	3	4	—	—
49–53	—	3	5	4	7	—	—
54–58	1	—	2	3	4	—	1
59–63	3	—	—	2	2	—	—
64–68	—	—	—	2	—	1	—
69–73	3	—	—	2	2	1	—
74–78	1	—	—	1	—	—	—
79–83	2	—	—	—	—	—	—
84–88	—	—	—	—	—	1	—

TABLE XVIII. MEAN LENGTHS (CM.) OF PRESUMED AGE CLASSES FROM F.R.S. *EXPLORER* DATA

Age classes ...	1	2	3	4	5
North Sea	—	32·5	59·7	72·0	81·5
N. Scotland	—	37	50·7	—	—
Barra-St Kilda	—	34·7	50·6	—	—
Minch	16·7	33·9	52·4	71·2	—
N.W. Ireland	14·4	32·2	49·9	66·7	—
Clyde	—	—	—	70·0	84
Irish Sea	—	—	58	—	—

TABLE XIX. PRESUMED YEARLY INCREMENTS (CM.) CALCULATED FROM TABLES XVIII AND IX

	2nd year	3rd year	4th year	5th year
From Table XVIII				
North Sea	—	27·2	12·3	9·5
N. Scotland	—	13·7	—	—
Barra-St Kilda	—	15·9	—	—
Minch	17·2	18·5	18·8	—
N.W. Ireland	17·8	17·7	16·8	—
Clyde	—	—	—	14·0
From Table IX				
Clyde	20·4	15·3	11·5	—

DISCUSSION

The results given in this paper, together with those of previous workers, suggest that the growth rate may be more variable than indicated by Hickling.

It seems that the methods of Birtwistle & Lewis were quite justified and that there are marked regional variations in the growth rate, though whether these are sufficient to be considered as 'variations ethnique' (Belloc, 1923) is open to doubt, since there is no evidence that there are not long-term variations among the same stock; indeed three authors have considered the fish from south of Ireland and have come to very different conclusions. The 'law' propounded by Belloc (1929) is that 'Le croissance du merlu varie avec la latitude. Ce poisson crôit d'autant plus rapidement qu'il vit à une latitude plus faible', and this he attributes to the water temperature, though the variations in the growth during individual years he attributes to the availability of suitable food. Hickling does not actually consider regional differences in the rate of growth, but regional differences in size at a given age, the southern fish being larger than the northern, and this he explains as being due to earlier spawning in the south. Apparent differences in growth rate in individual years were also noted, but were dismissed as being due to inconsistent sampling. A definite explanation of the fast growth rate shown in this paper cannot be given, and it can only be suggested that the rate of growth may change over a number of years. In view of this, and the recent revival of interest in the biology of the hake (see le Gall *et al.*, 1952) it is hoped to continue the present work in the Clyde area in an attempt to pick out any possible long-term growth-rate fluctuations.

SUMMARY

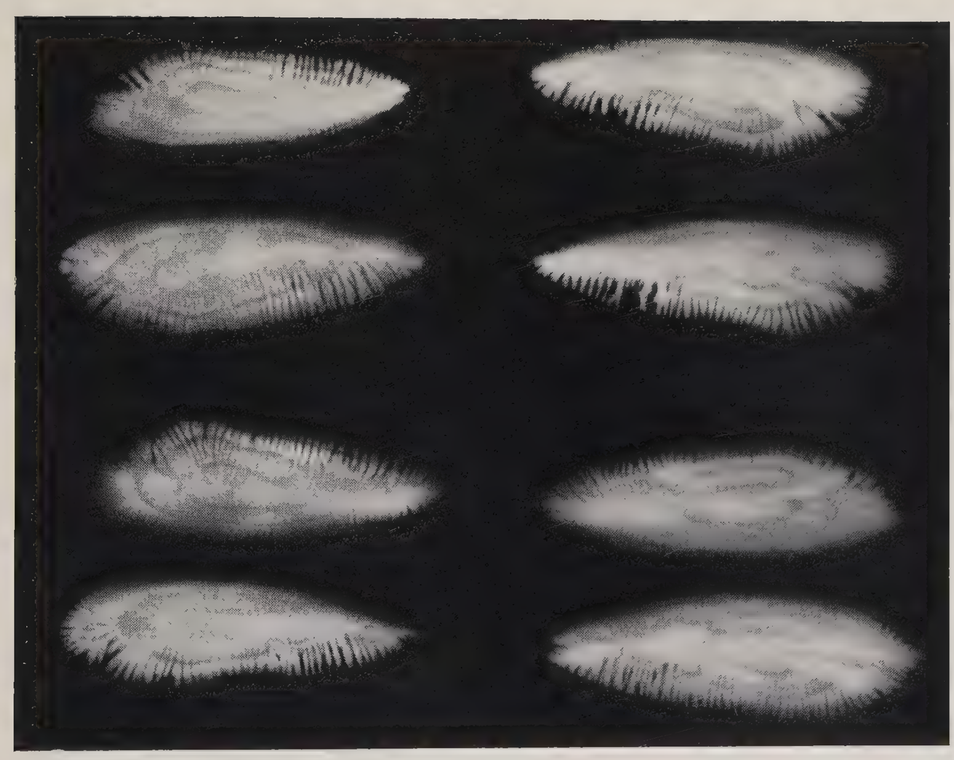
The growth rate of the hake found in the Clyde area has been studied from the inspection of the size-frequency distributions of trawl-caught fish and from an examination of 585 otoliths obtained during the winter of 1952–53. The results indicate that the annual increments decrease from over 20 cm. per annum during the first years to about 10 cm. at about 4 years of age.

These results are compared with those of other workers and found to be considerably greater than has been previously suggested; the nearest comparison being to those investigated in Manx water. It appears that the growth rate can vary considerably.

As reported by previous authors, the females have been found to grow faster than the males, though the difference does not become very pronounced until the third year of life.

The largest fish have been found in the deeper water of Loch Fyne, to the west of Arran and in Kilbrennan Sound, and this depth effect has also been noted elsewhere. The hake of all age-groups from the head of Loch Fyne have been found to be smaller than those off Tarbert, but except for these minor variations, the growth rate and size distribution has been found to be similar throughout the Clyde area.

A small amount of data on hake growth rate in other Scottish areas is given and these suggest that the growth rate noted in this paper is not confined to Clyde hake.



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EXPLANATION OF PLATE I

Otoliths of presumed three-year-old Clyde-caught hake. From top to bottom, left: ♂, 60 cm.; ♂, 63 cm.; ♀, 71 cm.; ♀, 71 cm.; right: ♀, 71 cm.; ♀, 70 cm.; ♀, 69 cm.; ♀, 72 cm.

J. Mar. biol. Ass. U.K. (1954), 33, 97–122
Printed in Great Britain

BREEDING AND GROWTH OF WHITING (*GADUS MERLANGUS* L.) IN ISLE OF MAN WATERS

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(Plate I and Text-figs. 1–6)

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INTRODUCTION

The whiting (*Gadus merlangus* L.) is an abundant food fish in waters to the west and south-west of the Isle of Man. As the stock is at times subject to intensive fishing it seemed desirable to study this species and possibly ascertain the effect of commercial exploitation on the biology of the fish.

The spawning period of the whiting in the Irish Sea has been investigated by Scott (1913, 1914) and Bal (1941); they deduced from counts of pelagic eggs that the spawning time extended from February to May or June, and that peak spawning occurred in April. The present investigation on seasonal changes in gonad condition of adult Manx whiting supports their conclusions.

Age and growth determinations have not previously been made on whiting from the Irish Sea.

This work was started at the suggestion of the late Prof. J. H. Orton, F.R.S., who gave much advice and encouragement. I am indebted to Dr J. W. Jones and Mr J. S. Colman for criticizing the manuscript.

MATERIAL AND METHODS

The whiting examined were caught between December 1948 and December 1951; few fish were taken between June and November in any one year. Three or four samples per month were taken in the seven months from December to June, which include the pre-spawning and main spawning period. The number of fish examined for gonad condition was over 3000, and the number aged (fish caught in 1950 and 1951) was a little over 2000, of which about half were '1 group' fish. The length-range of the fish sampled was 7–52 cm. and the age-range 0–8 years. Very few fish over 4 years old were taken, however.

The fish were taken by seine net and otter trawl. The cod-end mesh of the seine and one trawl was 1 in. bar, 4 in. circumference (Steven, 1950), another trawl had a cod-end mesh of $2\frac{1}{2}$ in. bar. The larger mesh was not used after March 1949 because it did not catch small whiting. In November 1950 a cover of shrimp netting, 90 rows per yard, was fitted to the cod-end of the trawl in use and thereafter only this net was used. The covered trawl effectively retained very small fish.

Hauls were made in depths between 20 and 44 fathoms (36·6–80·5 m.) to the west and south-west of the Isle of Man. The bottom deposit in 20 fathoms was sandy mud and shell, giving way to sandy mud and then soft mud as the depth increased.

Immature '0-group' fish were taken inshore by making hauls at night with a fine-meshed beach seine in Port Erin Bay in the south of the Isle of Man.

SEASONAL CHANGES IN GONAD CONDITION

Methods

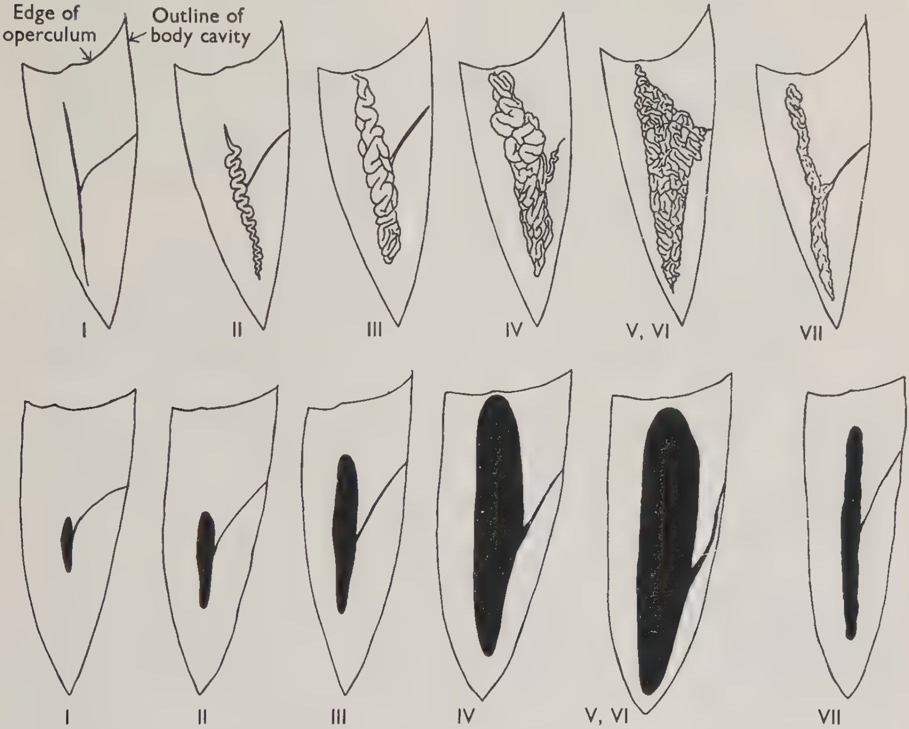
Trawl hauls were of short duration, and all the whiting from a haul were examined. When more than 600 fish were taken by seining, random samples of 100–200 fish were examined, otherwise complete catches were used. All fish were examined fresh, i.e. within 24 hr. of capture.

Each fish was measured to the nearest whole centimetre below its actual 'total length' (i.e. lengths from X cm. up to but not including $X+1$ cm. were all recorded as X cm.; the mid-point of the length group was taken as $X{\cdot}5$ cm.). Total length is defined as the length from the point of the upper jaw to the end of the longest fin ray of the tail fin, which was drawn into line with the main axis of the fish.

Sex was determined macroscopically and a 'gonad stage' assigned according to an arbitrary classification into seven stages, similar to that devised by Hjort (1910) for international herring research.

Classification of Gonad Condition

Bull (1928) used a five-stage maturity scale for whiting, modified from Graham's (1924) classification of cod gonads. Bull's classification has been amplified to avoid the use of intermediate stages, and extended to include a separate stage for newly spent gonads of both male and female whiting. The typical appearance of the gonad at each of the maturity stages employed in this investigation is described below. The relation between gonad size and size of the body cavity, and the shape of the gonad at each stage is shown in Text-Fig. 1.



Text-fig. 1. Changes in size of gonad in relation to the body cavity at different stages of maturity. Upper row: testis; lower row: ovary.

Stage	Male	Female
I	Immature virgin fish. Testis a very thin narrow translucent ribbon lying along an unbranched blood vessel	Immature, virgin fish. Ovaries very small, not more than one-fifth of the length of the body cavity, usually less than 2 cm. long. Elongated sausage shape. Whitish, translucent. Eggs microscopic
II	Maturing virgins or recovered spents in resting condition. Testis slightly lobed and lightly coiled. Opaque white at anterior end, transparent at posterior at first, later uniformly white	Maturing virgins or recovered spents in resting condition. Ovary not more than one-third length of body cavity, torpedo-shaped. Colour varying from wine-coloured, translucent, to dull orange
III	Maturing and ripening fish. Length of testis about three-quarters length of body cavity. Testis more strongly coiled with fat lobes, whitish grey in colour with blood vessels clearly seen in the lobes	Maturing and ripening fish. Ovary not more than one-half length of body cavity. Colour pink, pinkish buff, or flesh colour. Eggs opaque, visible to the naked eye in good light
IV	Maturing and ripening fish. Testis coiled and convoluted, completely opaque, white	Maturing and ripening fish. Ovary enlarged and distended occupies about two-thirds of body cavity. Orange pink in colour. Eggs clearly visible, opaque
V	Ripe fish not yet running. Testis a mass of tightly coiled and convoluted lobes. Completely opaque, creamy white in colour	Ripe fish not yet running. Ovary very swollen, tunica bursts easily. Some eggs transparent
VI	Running fish. Milt extruded easily by slight pressure on the flanks of the fish. Appearance as at stage V	Running fish. Eggs extruded easily by slight pressure on the flanks of the fish. Nearly all eggs transparent
VII	Newly spent fish. Testis crinkled and shrunken, rather bloodshot. Yellowish white colour	Newly spent fish. Ovary pinkish white, flaccid, shrunken, with some residual eggs

Gokhale (1953) used my classification as a basis for histological study of the whiting gonad. He showed that the macroscopic changes in the gonad are correlated with microscopic changes in the tissue. I am indebted to Dr Gokhale for permission to publish the following summary:

Stage	Microscopic Description of Tissue
	MALE
I {developing virgin} II {recovering spent}	Migrating germ cells and actively dividing spermatogonia, followed by a quiescent period
III (developing)	Few migrating germ cells. Mostly spermatogonia and a few spermatocytes
IV (maturing)	Number of spermatocytes very high; a few spermatozoa towards the centres of the lobules
V (full) VI (running)	Spermatogenesis complete; number of spermatozoa progressively greater
VII (spent)	Lobules almost empty; relict sperm being resorbed. Some migrating germ cells along the interlobular wall
	FEMALE
I {developing} II {virgin}	Large number of oogonia, presynaptic and post-synaptic oocytes
III (developing)	Oocytes larger: number of nucleoli increased. No oolemma in early stages but indications of oolemma forming in late stage III

IV (maturing)	Marked increase in size of oocytes. Yolk vesicles present in oocytes; oolemma present. Some immature ova undergoing degeneration
V (full) VI (running)	Eggs much grown. Some follicles empty; mature eggs lying in central cavity
VII (spent)	Few mature eggs remain, those present undergoing resorption; formation of corpora lutea. Large cavities in the tissue resulting from breakdown of interovular tissue. Loose granulosa cells and blood cells in tissue. Tunica thicker than previous stage
VII–II (spent-recovering)	Resorption of eggs nearly complete. Yellow patches as remnants of corpora lutea; formation of cell pearls. Oogonia and oocytes occupy the rest of the ovary. Tunica thick
II (recovered)	As virgin stage II, except that tunica thick

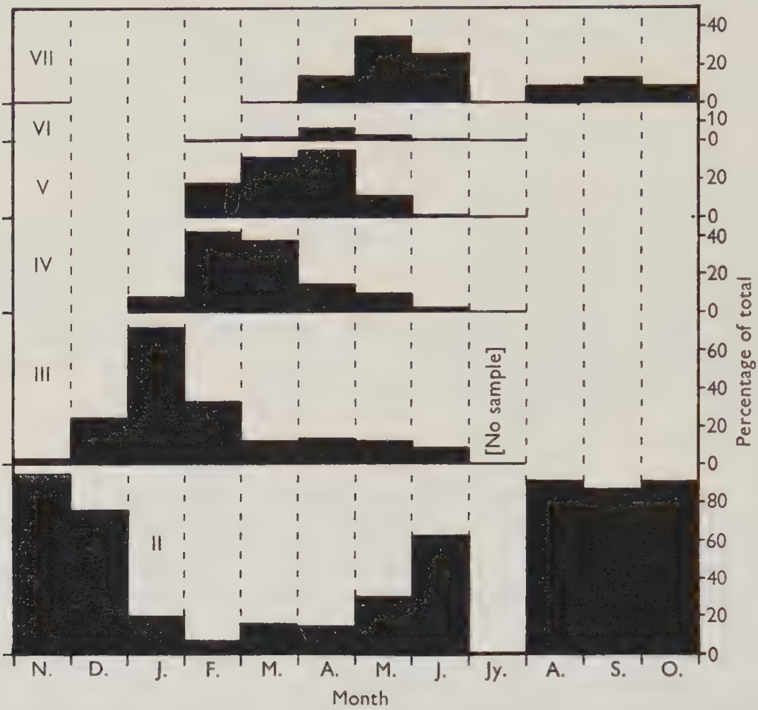
Gokhale's work showed that the spent-recovering and recovered spawner stages could have been distinguished from the developing virgin stage II in the female whiting, since the yellow patches described for stage VII–II are visible to the naked eye, and the thick tunica in stage II (recovered) can be felt between the fingers. No reliable macroscopic difference was found to distinguish maturing virgins from recovered spents in the male.

Seasonal Changes in Gonad Condition

Gonads were classified by their macroscopic appearance in each month except July, when no samples were available. Data are given in Table I and the seasonal changes observed are shown diagrammatically in Text-fig. 2. Immature virgin fish (stage I), which were found throughout the year, are not included in the table.

The records showed that some fish began to develop towards maturity as early as November, but the gonads of most fish were then in the recovered or resting state (stage II) and showed little or no change in size or appearance until January. From January until April there was a steady advance towards maturity, and in April ripe fish (stage V) were dominant. In May and June spent and recovering fish (stages VII and II) were the most common. Of the few fish examined from August to October the greater part were recovering or recovered from spawning, a few were spent, and none had maturing gonads. The large range in gonad condition in each month from February to June shows that there is wide variation in the time of the year at which different fish mature.

Running fish (stage VI) were taken between February and June inclusive; the spawning season thus extends over at least 5 months of the year. The small number of fish caught in gonad stage VI suggests that the actual spawning of an individual fish lasts only a short time, so that the chances of catching fish at this stage are low. The intensity of spawning in each month throughout the period is shown in Table II by a comparison of the number of ripe and running fish with the total number of potential spawners (i.e. fish with gonads developing towards maturity, or spent). Most of the fish spawn or are ready to spawn in March and April and spawning is virtually over by June. A very few spent fish, however, were found in the early winter (Table I), which suggests that there may be a small amount of late spawning.



Text-fig. 2. Monthly percentage of adult fish with gonads at each maturity stage.

Spent gonads appear to recover rapidly in the whiting, as by June most of the fish had gonads in the resting stage (II). This was reflected in the marked fall in the number of spent fish caught in June (Table I). 'Recovery' implies the complete resorption of residual eggs and sperm and the change in general appearance from the shrunken, rather bloodshot spent gonad to the firm, clear gonad described above for stage II.

Size and Age at First Maturity

The smallest Manx whiting found in an advanced state of maturity, i.e. stages V, VI, or VII, were a male of 19 cm. total length and a female of 21 cm.

No female fish in age-group 1 was found with gonads more mature than stage II. Ripe fish were commonly found in age-group 2; on the other hand, no immature virgin fish were found in age-group 3. It therefore appears that

TABLE I. NUMBER OF FISH IN EACH GONAD STAGE IN EACH MONTH (IMMATURE VIRGINS NOT INCLUDED)

(Figures in brackets are values derived from very small samples. The predominating gonad stage is in bold type.)

Month		Gonad stage II	III	IV	V	VI	VII	No. of fish examined
Nov.	No. of fish	64	2	—	—	—	1	67
	% of total	**95·5**	3	—	—	—	1·5	—
Dec.	No. of fish	40	13	—	—	—	—	53
	% of total	**75·5**	24·5	—	—	—	—	—
Jan.	No. of fish	19	68	7	—	—	—	94
	% of total	20	**72**	7·5	—	—	—	—
Feb.	No. of fish	16	73	92	39	1	—	221
	% of total	7	33	**41·5**	17·5	0·5	—	—
March	No. of fish	121	88	273	228	13	7	730
	% of total	16·5	12	**37·5**	**31**	2	1	—
Apr.	No. of fish	49	45	50	117	24	47	332
	% of total	15	13·5	15	**35**	7	14	—
May	No. of fish	130	51	44	48	12	144	429
	% of total	30	12	10	11	3	**33·5**	—
June	No. of fish	87	12	3	1	1	36	140
	% of total	**62**	8·5	2	1	1	25·5	—
July				No sample				
Aug.	No. of fish	10	—	—	—	—	1	11
	% of total	**(91)**	—	—	—	—	(9)	—
Sept.	No. of fish	20	—	—	—	—	3	23
	% of total	**(87)**	—	—	—	—	(13)	—
Oct.	No. of fish	10	—	—	—	—	1	11
	% of total	**(91)**	—	—	—	—	(9)	—

TABLE II. MONTHLY PERCENTAGE OF RIPE AND RUNNING FISH IN THE ADULT STOCK THROUGHOUT THE SPAWNING PERIOD

Month	Jan.	Feb.	Mar.	Apr.	May	June
No. of fish examined	94	221	730	332	429	140
No. of ripe and running fish	0	40	241	141	60	2
% of total	0	18	33	42·5	14	1·5

around the Isle of Man female whiting generally spawn for the first time at the end of their second year of life, and by the end of the third year (fish which are just entering age-group 3) all normal females are mature. Male whiting may spawn at the end of their first year of life: evidence of spawning or maturity was found in approximately 4% of the age-group 1 male fish taken during the spawning season. Practically all males of age-group 2 spawn.

Spawning Habits

No evidence was obtained of segregation of ripe fish into spawning shoals. From February until July fish in all stages of maturity were found together over all the area investigated. There are no records in the literature that special spawning shoals are formed. Ripe fish continue to feed.

The larger fish matured earlier in the season than the small fish of spawning size, and presumably spawned earlier (see Table III). In February and March, for example, the maturing females between 20 and 30 cm. long, most of them fish of age-group 2 ripening for the first time, were on average in a less advanced stage of gonad development than the females over 30 cm. long, most of which had spawned at least once before. Similar differences in time of ripening can be demonstrated among male fish of different length groups.

Records of gonad development in fish of known age suggest that fish in age-group 3 spawned earlier than did those in age-group 2, but the evidence is not conclusive; it would appear that size is more important than age in determining the time of maturation.

TABLE III. NUMBER OF FISH AT EACH STAGE OF GONAD MATURITY, SHOWN FOR DIFFERENT LENGTH-GROUPS

(Data for February and March 1949 and 1950. The most common stage of maturity is shown in heavy type.)

		Gonad stage						No. of fish
		II	III	IV	V	VI	VII	
% of female fish at each stage in Feb.	length 20–30 cm.	14	**54**	25	7	0	0	72
	length >30 cm.	0	28·5	**51**	20·5	0	0	49
% of female fish at each stage in Mar.	length 20–30 cm.	24	25	**26**	23	1	0·5	181
	length >30 cm.	1	5·5	30	**61**	1	1·5	124

Sex Ratio

Of 3118 fish sexed, of length range 12–52 cm., 52·8% were male, 47·2% female. Fulton (1892) found that 32·2% of the whiting he examined were male, 67·8% female. The average size of Fulton's fish was greater than that of mine, and presumably he had more large fish; it will be shown later that the larger fish are predominantly female.

Abnormalities in Gonads

Two female fish were observed, each with one ovary developing normally and the other atrophied. One fish was 27 cm. long with the normal ovary in gonad stage III, the other 30 cm. long with the normal ovary ripe, gonad stage V. No internal parasites which might have caused the abnormality were found in either fish.

Hermaphrodite whiting are rare (Desbrosses, 1948): none were seen among 3118 Manx whiting.

Spawning of Whiting in Different Regions

The spawning behaviour of the Manx whiting is intermediate between that of fish from the northern part of the geographical range of the species and fish from the southern part of the range. This is shown in relation to the spawning season, the time of maximum spawning activity, and age and length at first maturity.

Spawning of the whiting occurs progressively later as the latitude increases. Table IV shows that there is a variation of 4 months in the onset of spawning in different regions of western Europe. Desbrosses (1945) showed that variation in spawning time is closely connected with sea temperature; in the Irish Sea gonad development is greatest when sea temperatures are low, in February and March, and spawning is at a maximum in April when sea temperature starts to rise. From Table IV it appears that in many areas maximum spawning occurs as the temperature rises after the winter minimum.

Age and length at maturity increase from south to north of the species range. The data for Manx whiting agree closely with those for Scottish fish (Fulton, 1892, 1901) and are a good fit in a table prepared by Desbrosses (1945) to show the geographical variation.

AGE AND GROWTH

Methods

Age was determined by means of otoliths, of which less than 1 % had to be rejected as unreadable. Some scales were also examined, but the interpretation of many of these was doubtful because secondary growth rings were common and difficult to distinguish from true growth rings.

From March 1950, otoliths were taken from the whiting caught for the study of gonads. Adequate samples of offshore fish were obtained from March to June 1950, and from January to June 1951. Most of the samples were taken at a time of year when there is little growth in length of the fish.

Fresh fish were measured and sexed as described on p. 98. Otoliths were then removed in the following way: the fish was laid on its side, the blade of a strong scalpel was slipped under the operculum and drawn forward dorsally towards the point of the snout, thereby cutting through the skull. The otolith thus exposed was removed, undamaged, by means of forceps. It was found convenient to remove both large otoliths and place those from each fish in a separate labelled envelope. The envelopes containing otoliths from each sample were stored together in a sealed jar of spirit.

Otoliths were prepared for examination by removal of the remains of the sacculus (if present), followed by a wash in clean 95 % spirit and immersion for a few minutes in pure creosote until the rings showed clearly (Johnston, 1938). Thick otoliths were found to be more easily read after the convex surface had been ground slightly with carborundum (Menon, 1950).

The otoliths were examined in a matt black dish by reflected light. It was found that any magnification confused rather than clarified the reading (cf. Hickling, 1933), and all age assessments were made after examination with the naked eye.

Table IV. Spawning Period of the Whiting in Different Localities

Region	Approx. latitude	Authority	Method of assessment	Extent of spawning period	Month of maximum spawning	Mean sea temp. at time of max. spawning (° C.)	Annual range of sea temperature (° C.)
Iceland	63–65° N.	Schmidt (1909)	Running fish and presence of eggs	May (or earlier)–Mid July	—	8 (June)	Feb. 5–6° to Aug. 10°
Iceland	63–65° N.	Ehrenbaum (1936)	Presence of eggs	—	End of May	7·5	Feb. 5–6° to Aug. 10°
Shetland	60–61° N.	Damas (1909)	Eggs and young larvae	Up to Sept.	—	—	Feb. 6° to Aug. 12°
Scotland	56–58° N.	Fulton (1892)	Ripe fish	Mar.–Aug.	Apr.	6	Feb. 5° to Aug. 12°
Scotland, E. (Firth of Forth)	56° N.	Williamson (1895)	Abundance of eggs	Mar.–June	Apr.	6	Mar. 5° to Aug. 12°
Scotland, W. (Firth of Clyde)	56° N.	Williamson (1899)	Abundance of eggs	Feb.–Aug.	Apr.	8	Feb. 7° to Aug. 12°
Ireland, W.	52–56° N.	Holt (1891, 1893)	Abundance of eggs	Mar.–June	Mar., Apr.	9	Feb. 9° to Aug. 14″
Irish Sea (Manx Waters)	54° N.	Scott (1913, 1914)	Abundance of eggs	Feb.–May	Early Apr.	9	Mar. 7° to Aug. 14°
Irish Sea (Manx Waters)	54° N.	Bal (1941)	Abundance of eggs	Feb.–June	Apr.	9	
Irish Sea (Manx Waters)	54° N.	Present investigation	Ripe and running fish	Feb.–June	Apr.	9	
Heligoland	54° N.	Heinke & Ehrenbaum (1900)	Abundance of eggs	Jan.–July	Up to May	9·5 (May)	Feb. 3° to Aug. 16·5°
Heligoland	54° N.	Heinke (1905)	Running fish Abundance of eggs	Mar.–June Jan.–May	Mar., Apr., May	3·5, 6, 9·5	
Plymouth area	50° N.	Cunningham (1889)	Presence of eggs	Feb. onward	—	—	Mar. 8·5° to Aug. 15·5°
Plymouth area	50° N.	Clark (1920)	Abundance of larvae	Feb.–July	—	—	
French Atlantic coast	46·5–48° N.	Desbrosses (1945)	Running fish	Jan.–June	Feb., Mar.	10, 9·5	Mar. 9·5° to Aug. 15·5°

Temperature data from Bureau du Conseil (1933), Danish Met. Institute (1917), Proudman, Lewis & Dennis (1937).

Description of the Whiting Otolith

The otolith of the whiting is an elongate structure, rounded or obliquely truncated anteriorly and tapering to a point posteriorly. The shape changes considerably with age (see Pl. I). The margin is crenellated. The surface which lies towards the brain is slightly convex in transverse section; the outer surface is flattened. Whiting otoliths are thinner and flatter than those of most other gadoids (Scott, 1906).

When examined against a matt black background by reflected light, alternate light (opaque) and dark (translucent) concentric zones can be seen (Pl. I). The core of the otolith may be translucent or opaque, and outside this is an opaque zone in which one or more very narrow translucent bands are often visible. This zone is bounded by a clearly marked, though often complex, translucent band: this will be called the first band and the region inside it called the centre. Alternate opaque zones and translucent bands are visible outside the first band up to a total of seven of each. The larger the fish, the greater is the number of zones and bands which may be seen.

The periphery may be formed of an opaque zone or a translucent band, dependent largely on the season of capture. Since the edge may be very thin, it is sometimes difficult to distinguish whether in fact the edge is opaque or translucent (Wallace, 1907; Jones & Hynes, 1950), but with practice it becomes possible.

The relative width of the translucent bands and opaque zones varies considerably in otoliths from different fish (see Pl. I B), but when there are more than three bands on an otolith these bands are narrower and more crowded towards the edge.

The two large otoliths from the same fish nearly always have a similar appearance and give identical readings; about 1% of the total number of fish examined had one typical otolith and one completely crystalline, showing no alternating bands and zones. In one male fish, 33 cm. long, the left otolith was larger than the right, and had at the edge a translucent band that was not present on the right otolith. This was the only discrepancy of the kind in 1088 comparisons of this nature.

Interpretation of Seasonal Changes in the Otolith

The otoliths of 0-group fish taken in July are completely opaque or show a small translucent core with an opaque peripheral area, i.e. only the centre of the otolith has been laid down. In August most of the otoliths show a very narrow translucent edge. Further growth takes place by the addition of opaque material so that the otolith shows a central core bounded by a translucent line, and an opaque edge. The translucent line may become overlaid in older otoliths but can be revealed by grinding the convex surface of the otolith.

The translucent band recognized as the first annual band is broader than the translucent line formed in August, and is more clearly marked, although it may be of a double or complex nature, i.e. there may be one or two opaque lines within the translucent band. The annual band is laid down at the edge of the otolith in February, March and April (cf. Desbrosses, 1948). By June most of the otoliths show new growth, at the edge of the otolith, which is opaque. Thus the first annual band is formed when the fish has lived one summer and one winter after hatching, and is complete when the fish is about 1 year old. The actual age of fish in the same age-group may, however, vary by several months because there is a long spawning period, which means that a fish hatched early in the season is already 4 months old by the time the last spawned eggs hatch.

The second and subsequent translucent bands are usually laid down at the edge of the otolith between January and May. Otoliths may have a translucent edge in any month of the year but there is a well-marked maximum in February and March. Thompson (1926) found that the 'winter ring' of narrow sclerites on scales was laid down in February and that new growth started in June; this shows some correspondence with the state of the otolith.

Age estimates were made from the number of bands and zones on the otolith as follows:

Otolith with no translucent band	Age-group 0
Otolith showing centre and translucent edge	Age-group 1
Otolith showing centre, translucent band and opaque edge	Age-group 1
Otolith showing centre, translucent band, opaque zone and translucent edge	Age-group 2
Otolith showing centre, two translucent bands with opaque zone between, and opaque edge	Age-group 2

and so on up to age-group 8. In short, the fish was allotted to the age-group corresponding to the number of translucent bands seen on the otolith, irrespective of whether the outermost translucent band was at the edge or within an opaque edge.

Age and Growth as shown by Otolith Readings

Age determinations made by means of otolith readings showed that fish in all age-groups up to 8 were present in the area investigated. Age-groups 1 to 3 were well represented, but older fish were scarce. Ellis (1950) has suggested that older whiting in the North Sea migrate to deep water. A similar migration might account for the scarcity of fish over 3 years old in the samples examined; I have no evidence on this point, since this investigation was limited to depths of 20–45 fathoms.

The oldest female fish was 52·5 cm. long and belonged to age-group 8. The largest male was 47·6 cm. long but the otolith was not readable beyond the seventh band because the outermost markings were confused and indistinct; the fish was certainly more than 7 years old. Four males and four females in age-group 7 were taken. It is possible that older fish would be found in deep water, but the age limit of 8 years agrees fairly closely with the observations of Saemundsson (1925), Desbrosses (1948) and Knudsen (1950).

The length distribution of the fish in each age-group is shown in Text-fig. 3. Numerical data are given separately for the two years 1950 and 1951 in the Appendix. The range in length and the mean length at each age-group are shown in Table V. Mean lengths at age-groups 5, 6 and 7 are probably not very reliable because the number of fish in these groups was small.

TABLE V. MEAN LENGTH AND LENGTH RANGE OF WHITING AT EACH AGE-GROUP IN 1950 AND 1951

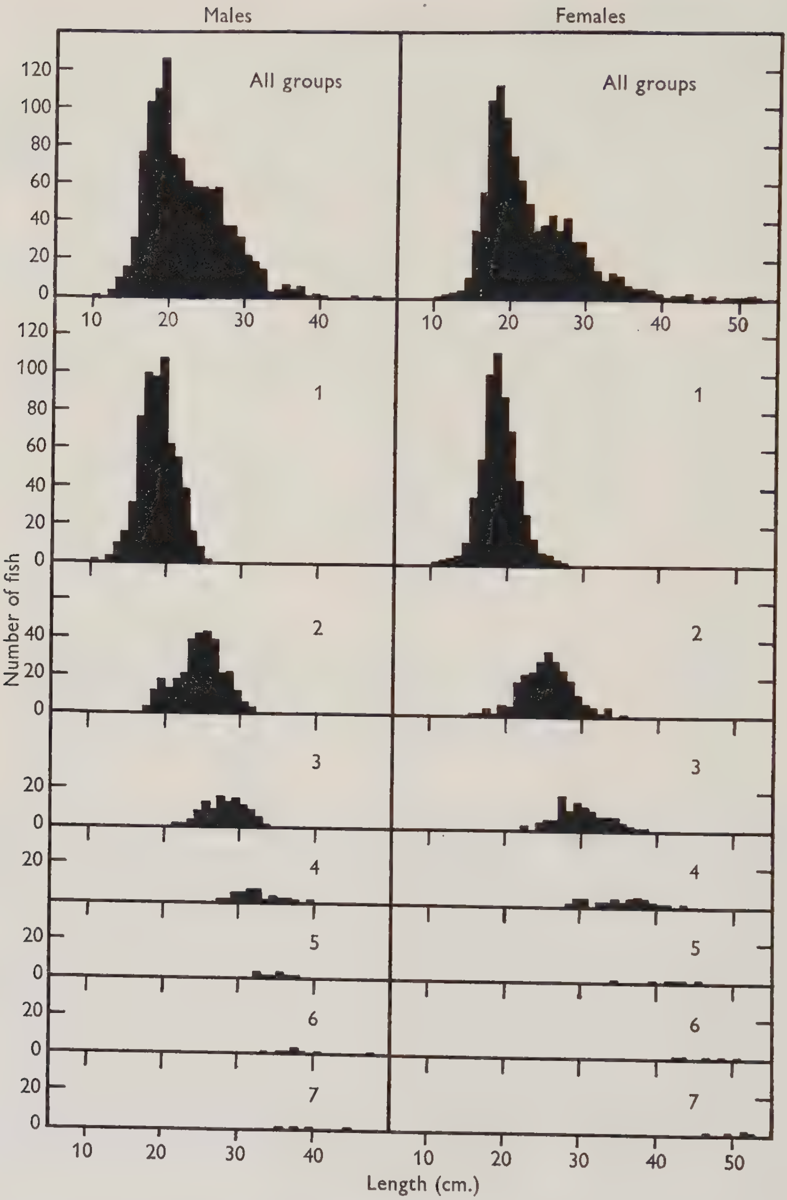
(Age determined by otolith readings.)

Age-group	No. of fish examined	Range in length (cm.)	Mean length (cm.)	Increase per year (cm.)
		Male		
1	525	10–26	18·82	—
2	322	17–32	24·62	5·8
3	105	21–34	28·08	3·5
4	35	27–40	32·3	4·1
5	10	32–38	34·6	2·4
6	9	33–48	38·3	3·7
7	4	35–46	39·3	1·0
		Female		
1	578	10–28	18·86	—
2	245	15–36	25·35	6·5
3	110	22–39	30·0	4·7
4	37	28–44	35·2	5·2
5	6	34–46	41·1	5·9
6	5	42–51	46·3	5·2
7	4	46–52	49·8	3·5
8	1	—	52·5	2·7

Growth is rapid in both sexes for the first year, and the mean length at the end of the year is between 18 and 19 cm. The range in length is very great. Both male and female fish grow about 6 cm. in the second year. Too few old fish were caught to make an accurate assessment of growth beyond age-group 4, but the available data suggest that the females continue to grow steadily at a rate of 5 or 6 cm. per year until the seventh year (age-group 6) when growth slows down. The male fish grow less than the females after the second year, and the disparity in the mean lengths of the male and female fish at each age-group increases with age (see Text-fig. 4). Nearly all fish examined of more than 40 cm. total length were females (Text-fig. 3). Similar sexual differences in growth rate have been demonstrated in Icelandic and French Atlantic whiting (Saemundsson, 1925; Desbrosses, 1948).

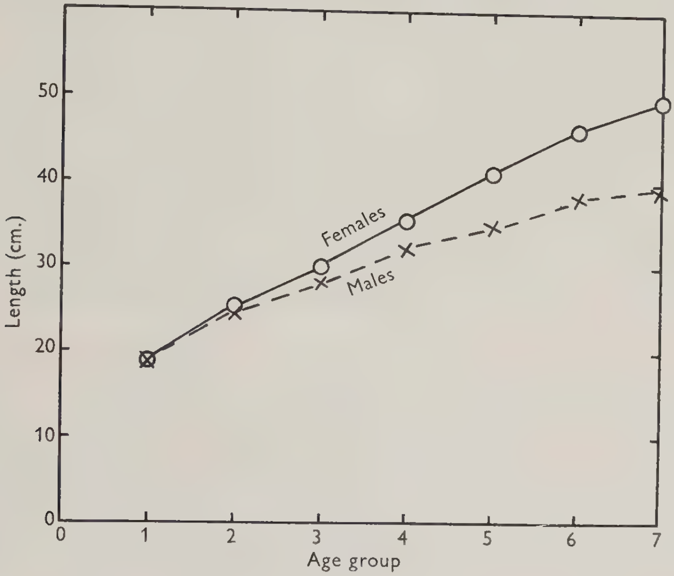
DISCUSSION

The age determinations from otolith readings were based on the assumption that growth rings on the otolith are laid down annually. There is no absolute proof available that this is true (Graham, 1928); seasonal records of the state

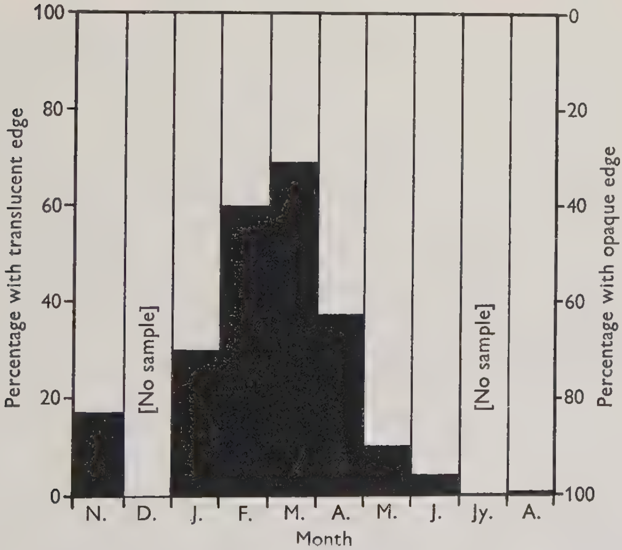


Text-fig. 3. Age and length distribution of offshore whiting, 1950 and 1951. Age determined by otolith readings. The numbers 1–7 refer to the different age-groups.

of the edge of the otolith were incomplete. Records made in 1950 are shown in Text-fig. 5; those for 1951 showed a very similar pattern. The number of otoliths with a translucent edge (i.e. those in the course of laying down a translucent year band) shows a peak in February and March when the sea temperature around the Isle of Man is lowest, but there is no complete change-over



Text-fig. 4. Mean length at each age-group, 1950 and 1951.



Text-fig. 5. Condition of margin of otolith through the year. Age-groups 2–7.

from translucent edge to opaque edge, data are available for only seven months of the year, and the number of specimens examined in November and August was small. The possibility that two translucent bands are laid down in one year cannot be completely excluded, although it appears unlikely.

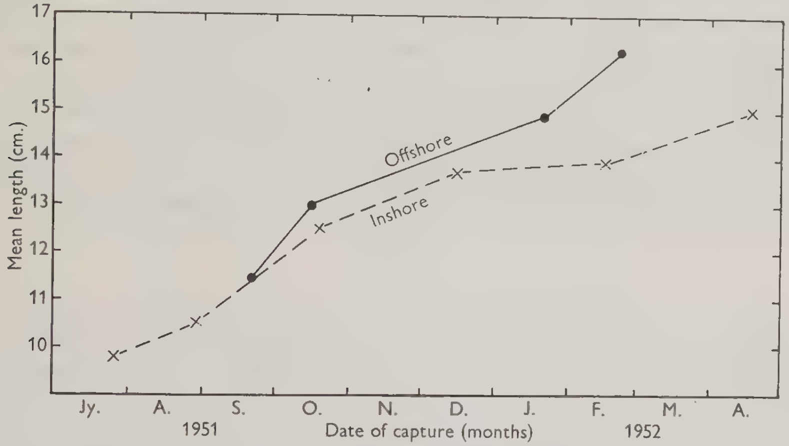
The form of the histograms for length distribution shown in Text-fig. 3 suggests that some estimates of age from otolith readings may be wrong. Transfer between adjacent groups of a few of the data (e.g. for males, length 18 and 19 cm. from group 2 to group 1, females length 27 cm. from group 3 to group 2) would make each group more nearly normal in length distribution. Errors in otolith readings could have arisen where secondary bands and true year bands were misinterpreted; such errors do not appear to be numerous and probably have little effect on the calculated mean lengths of each age-group.

Interpretation of the length-frequency diagrams by Petersen's (1895) method is difficult because the whiting has a long spawning period; consequently the length range of a year-class is large and there is a considerable overlap of length ranges of different year-classes. A fish 30 cm. long, for example, may be 2, 3 or 4 years old. In such circumstances the method is not very reliable (Graham, 1928). It can be seen from Table VII (Appendix) that modal lengths from the total number of fish correspond in only a few instances with the mean lengths of year-groups determined by otolith readings.

It is probable that the samples examined were not entirely representative of the whiting population in the area because of mesh selection by the nets used, and selection of habitat by fish of different ages. No special precautions were taken in the early months of 1950 to prevent the escape of small fish through the meshes of the net, and comparison of the length-frequency table for age-group 1 fish caught in 1950 with that for 1951 (Table VII) suggests that mesh selection did occur in 1950. It was noticeable that the smallest whiting were present in the seine net when the total catch of fish of all species was large, in which case it would be more difficult for fish to escape from the cod end. The fine mesh fitted to the gear used throughout 1951 prevented the escape of small fish, but may have affected the fishing capacity of the net so as to reduce the number of large fish caught. Investigations on this point are proceeding but not complete. Davis (1934) states that his experiments show clearly that no trawl net will give a true sample of the entire population on any ground.

There is some evidence of selection of habitat by the 0-group and 1-group fish. Comparison of the mean lengths of inshore and offshore catches of the 1951 brood of whiting (Text-fig. 6) shows that the offshore fish were larger than those inshore. Great care was taken to avoid mesh selection by the gear used, and samples were numerically large. Scattered observations in earlier years fit well with the 1951–52 data shown in the figure (unpublished work at

Port Erin). Most of the small 1-group whiting caught offshore in spring were at the shallowest limit of the fishing ground (20–25 fathoms). 1-group fish appeared sporadically in sandy bays inshore throughout the summer and autumn. The area between the bays and the offshore fishing ground is too rough for net fishing and there is therefore insufficient evidence to assess the relation between the depth of water and the size of the 1-group fish, or to determine the movements of the fish. The relation and interchange between the inshore shoals and the offshore stock is complex and requires more study, but it seems likely that there is a movement of some young fish away from inshore waters throughout the winter months from September onwards. The offshore stock is probably recruited gradually from the inshore shoals; and the larger fish move offshore in greater numbers than do the smaller fish.



Text-fig. 6. Mean length of inshore and offshore whiting of 1951 brood.

Old fish (age-groups 5–7) were usually taken in the deepest water fished (40–45 fathoms), and the largest whiting are most commonly caught by local fishermen in deeper water. Ellis (1950) found a migration of older whiting to deep water in the North Sea. Thus the largest fish of the higher age-groups may not have been represented in the samples examined, and the calculated mean length for these age-groups may be too low.

The graphs (Text-fig. 4), expressing the increase in length of whiting with age, are based on data from samples collected in the two years 1950 and 1951. A more accurate and direct statement of the growth of the fish would be obtained by sampling given year-classes in each year throughout their life span; this would involve a long-term programme. Short-term investigations, such as the present one, cannot give due weight to annual variations in growth; such variations, particularly in the growth of 0-group fish (Knudsen, 1950), are probably the cause of anomalies in growth calculations from age analysis. Table V, for example, shows irregularities in the annual increase in length, particularly between age-groups 2 to 3 and 3 to 4, and similar irregularities are shown in the data of Damas (1909) and Desbrosses (1948).

The interpretation of Text-fig. 4 (p. 111) as a growth curve of whiting in the Irish Sea must therefore be made with reservation; variations from the curve are to be expected if data are collected in different years, and if the fish examined are caught at different depths.

The results of previous work on age and growth in the whiting are set out in Table VI. Direct comparisons between investigations are unjustified, since few workers have shown separate growth rates for male and female fish, and samples were taken in different seasons, different years, and from different depths; all these factors may affect the length of fish at different ages (Desbrosses, 1948; Knudsen, 1950; Damas, 1909). It is clear, however, that Icelandic fish grow faster than those of other regions, and that whiting from Denmark, the North Sea and the Irish Sea have similar growth rates over the first 4 years. The discrepancy between Fulton's (1901) figures and those of other workers suggests that Fulton's higher length series, separated by Petersen's method, were composed of more than one age-group.

SUMMARY

The macroscopic seasonal changes in the gonads and the age and growth of whiting, caught in the Irish Sea near the Isle of Man, have been investigated.

Seasonal changes in gonad condition were assessed by applying an arbitrary classification of maturation into seven stages which are described.

Development of gonads from a 'resting' state to the 'full' (ripe) condition takes 3–4 months.

The whiting of this area have a long spawning period extending from February to June, with a peak in April.

Recovery from spawning is rapid.

The male whiting of this area may spawn for the first time at the end of their first year, but most fish spawn for the first time at the end of their second year or beginning of their third year of life, i.e. in age-group 2. Nearly all age-group 3 fish spawn.

The smallest spawning fish found were a male of 19 cm. and a female of 21 cm. length.

Few abnormalities in gonads were found.

Age determinations were made by means of otolith readings.

The translucent year-band in the otolith is laid down in age-group 1 fish in February, March or April, and in older fish between January and May.

TABLE VI. LENGTH WITH AGE OF WHITING FROM DIFFERENT LOCALITIES

Region	Authority	Method of age determination	Months in which samples taken	Mean length in cm. at each age-group (figures in brackets based on less than 5 fish)														
				1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8
Iceland	Saemundsson (1925)	Scales	Aug.	18·1	—	27·0	—	39·3	—	46·6	—	51·4	—	54·7	—	59·2	—	(62·5)
S. Norway	Damas (1909)	Measurements	Sept.	—	24	—	—	—	—	—	—	—	—	—	—	—	—	—
Skagerrak	Nybelin (1946 unpublished) (cited by Desbrosses)	Otoliths	Mar.	18·9	—	25·4	—	28·7	—	33	—	—	—	—	—	—	—	—
N. Denmark	Knudsen (1950)	Measurements, scales and otoliths	July	17·5	—	24·2	—	29·9	—	—	—	—	—	—	—	—	—	—
S. Denmark	Knudsen (1950)	Measurements, scales and otoliths	July	17·3	—	24·6	—	29·9	—	32·5	—	—	—	—	—	—	—	—
North Sea 54–59° N.	Damas (1909)	Scales and otoliths	Nov.	—	20·2	—	23·9	—	30·0	—	33·7	—	37·3	—	(39)	—	—	—
N. North Sea	Carr (1909)	Scales	Mar.	—	—	25·6	—	27·9	—	29·6	—	—	—	—	—	—	—	—
N. North Sea	Fulton (1901, 1902)	Measurements	Oct.–Dec.	—	23·7	—	31·3	—	46·9	—	53·4	—	—	—	—	—	—	—
N. North Sea	Thompson (1926)	Scales	—	13·5	—	21·5	—	28	—	—	—	—	—	—	—	—	—	—
Irish Sea	This investigation	Otoliths	Spring (Jan.–June)	♂ 18·8	—	24·6	—	28·1	—	32·2	—	34·6	—	38·3	—	(39·3)	—	—
				♀ 18·9	—	25·4	—	30·0	—	35·2	—	41·1	—	46·3	—	(49·8)	—	(52·5)
English Channel	Thomson (1904)	Scales	Winter	7–18	—	29–33	—	32–42	—	34–46	—	49	—	—	—	—	—	—
Gulf of Gascony	Desbrosses (1948)	Otoliths	Winter	♂ 18·1	—	23·4	—	30·3	—	40	—	(38·2)	—	(46)	—	—	—	—
				♀ 19·0	—	26·7	—	35·2	—	45·6	—	49·3	—	(59)	—	—	—	—

The maximum age of the fish examined was 8 years.

The offshore stock in the area investigated consisted mainly of fish in age-groups 1, 2, and 3, with lengths lying between 15 and 35 cm.

There is a difference in growth rate between male and female fish; the females grow faster and reach a greater maximum length.

Both male and female fish reach a length of about 19 cm. at the end of the first year. Thereafter female whiting increase in length 5 or 6 cm. per year for five years, after which the rate of growth slows down. Males grow about 6 cm. in their second year; the rate of growth then declines and is very small by the sixth year.

The limitations of the methods employed are discussed.

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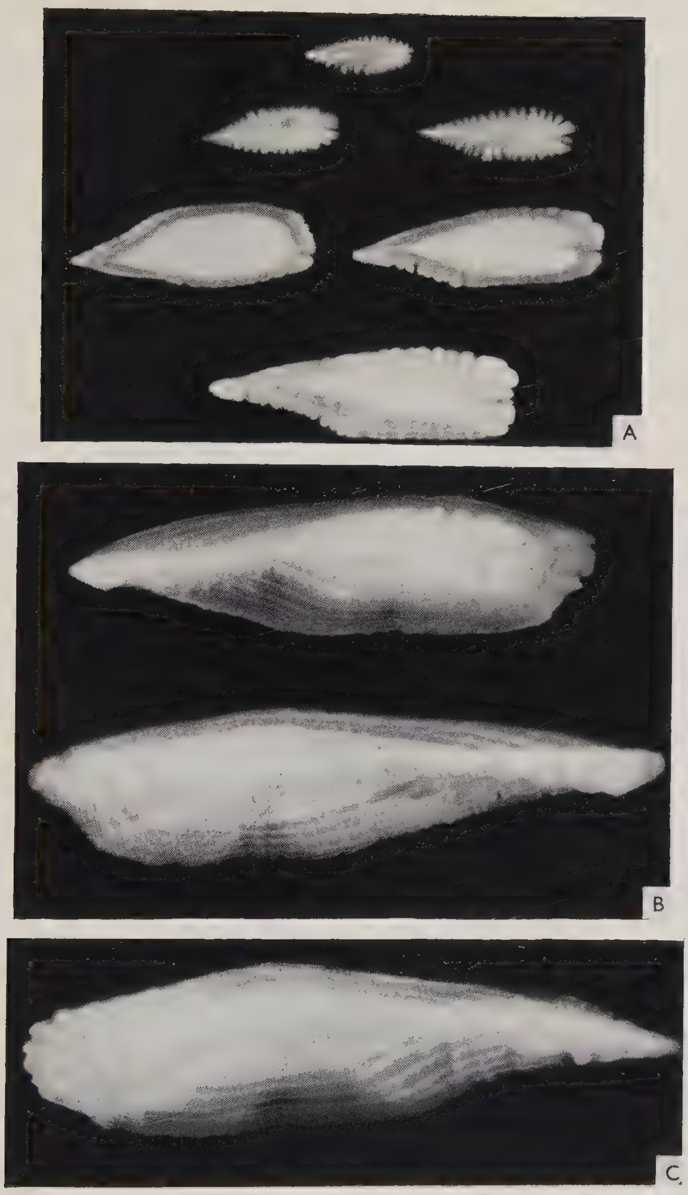
EXPLANATION OF PLATE I

Otoliths of whiting ×3. A, top to bottom: ♂, 9 cm., July 1950, centre laid down; two ♂♂, 11 cm., August 1950, new growth outside centre; ♂, 18 cm., May 1950, concave and convex surfaces; ♂, 22 cm., May 1950, showing centre and first year band of extreme complexity. B: ♂, 39 cm., age-group 7 showing broad translucent bands with some 'doubling' of bands; ♀, 50 cm., age-group 6, a thick otolith with a keel-like process at the posterior end. C: ♀, 49 cm., age-group 7 showing centre, seven translucent year bands and a narrow opaque edge; the translucent bands show dark.


A
B
C

APPENDIX

TABLE VII. NUMBER AND LENGTH OF FISH IN EACH AGE-GROUP 1950 AND 1951

Length (cm.)	No. of fish in each age-group							Total
	1	2	3	4	5	6	7	
				♂ Whiting, 1950				
12	1	—	—	—	—	—	—	1
13	3	—	—	—	—	—	—	3
14	6	—	—	—	—	—	—	6
15	13	—	—	—	—	—	—	13
16	27	—	—	—	—	—	—	27
17	45	—	—	—	—	—	—	45
18	52	5	—	—	—	—	—	57
19	52	11	—	—	—	—	—	63
20	32	9	—	—	—	—	—	41
21	23	13	—	—	—	—	—	35
22	22	10	—	—	—	—	—	32
23	9	16	1	—	—	—	—	26
24	4	19	—	—	—	—	—	23
25	—	21	8	—	—	—	—	29
26	—	17	4	—	—	—	—	21
27	—	10	9	—	—	—	—	19
28	—	17	10	—	—	—	—	27
29	—	8	8	2	—	—	—	18
30	—	5	7	2	—	—	—	14
31	—	2	9	6	—	—	—	17
32	—	—	3	6	3	—	—	12
33	—	—	—	—	—	1	—	1
34	—	—	—	3	—	—	—	3
35	—	—	—	1	3	1	1	6
36	—	—	—	2	1	—	—	3
37	—	—	—	1	1	3	1	6
38	—	—	—	—	—	1	—	1
39	—	—	—	1	—	—	1	2
40	—	—	—	—	—	1	—	1
Total	289	163	59	24	8	7	3	553
Mean length	19·05	24·75	28·75	33·0	34·75	37·2	37·5	

TABLE VII (*cont.*)

Length (cm.)	No. of fish in each age-group 1	2	3	4	5	6	7	Total
				♀ Whiting, 1950				
12	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—
14	4	—	—	—	—	—	—	4
15	12	—	—	—	—	—	—	12
16	14	—	—	—	—	—	—	14
17	41	4	—	—	—	—	—	45
18	55	—	—	—	—	—	—	55
19	37	4	—	—	—	—	—	41
20	30	2	—	—	—	—	—	32
21	25	12	—	—	—	—	—	37
22	11	14	1	—	—	—	—	26
23	10	13	—	—	—	—	—	23
24	4	16	1	—	—	—	—	21
25	4	14	2	—	—	—	—	20
26	—	11	1	—	—	—	—	12
27	—	6	5	—	—	—	—	11
28	—	10	4	—	—	—	—	14
29	—	5	7	2	—	—	—	14
30	—	3	7	2	—	—	—	12
31	—	1	6	—	—	—	—	7
32	—	1	5	1	—	—	—	7
33	—	3	2	1	—	—	—	6
34	—	—	6	4	1	—	—	11
35	—	1	2	2	—	—	—	5
36	—	—	2	4	—	—	—	6
37	—	—	—	4	—	—	—	4
38	—	—	1	4	—	—	—	5
39	—	—	—	2	1	—	—	3
40	—	—	—	1	—	—	—	1
41	—	—	—	—	—	—	—	—
42	—	—	—	—	1	1	—	2
43	—	—	—	1	1	1	—	3
44	—	—	—	—	—	—	—	—
45	—	—	—	—	1	—	—	1
46	—	—	—	—	—	1	1	2
47	—	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—	—
49	—	—	—	—	—	—	1	1
50	—	—	—	—	—	1	—	1
51	—	—	—	—	—	—	2	2
52	—	—	—	—	—	—	—	1 (8 yr.)
Total	247	120	52	28	5	4	4	461
Mean length	19·30	24·98	30·85	36·0	41·1	45·75	49·75	

Length (cm.)	No. of fish in each age-group 1	2	3	4	5	6	7	Total
	♂ Whiting, 1951							
10	1	—	—	—	—	—	—	1
11	—	—	—	—	—	—	—	—
12	2	—	—	—	—	—	—	2
13	7	—	—	—	—	—	—	7
14	10	—	—	—	—	—	—	10
15	18	—	—	—	—	—	—	18
16	50	—	—	—	—	—	—	50
17	55	3	—	—	—	—	—	58
18	46	7	—	—	—	—	—	53
19	56	7	—	—	—	—	—	63
20	30	4	—	—	—	—	—	34
21	32	5	1	—	—	—	—	38
22	17	11	1	—	—	—	—	29
23	7	23	2	—	—	—	—	32
24	4	23	8	—	—	—	—	35
25	1	22	5	—	—	—	—	28
26	—	32	5	—	—	—	—	37
27	—	11	7	1	—	—	—	19
28	—	5	3	1	—	—	—	9
29	—	4	7	3	—	—	—	14
30	—	1	4	3	—	—	—	8
31	—	1	—	1	—	—	—	2
32	—	—	2	1	—	—	—	3
33	—	—	1	—	1	—	—	2
34	—	—	—	—	1	—	—	1
35	—	—	—	1	—	—	—	1
36	—	—	—	—	—	1	—	1
37	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—
41	—	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—	—
43	—	—	—	—	—	—	—	—
44	—	—	—	—	—	—	1	1
45	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—
47	—	—	—	—	—	1	—	1
48	—	—	—	—	—	—	—	—
49	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—
Total	336	159	46	11	2	2	1	557
Mean length	18·63	24·49	27·22	30·5	34	42	44	
Mean length 1950+1951	18·82	24·62	28·08	32·24	34·6	38·3	39·25	

TABLE VII (*cont.*)

Length (cm.)	No. of fish in each age-group							Total
	1	2	3	4	5	6	7	
			♀ Whiting, 1951					
10	1	—	—	—	—	—	—	1
11	2	—	—	—	—	—	—	2
12	3	—	—	—	—	—	—	3
13	4	—	—	—	—	—	—	4
14	7	—	—	—	—	—	—	7
15	23	1	—	—	—	—	—	24
16	41	1	—	—	—	—	—	42
17	59	—	—	—	—	—	—	59
18	56	1	—	—	—	—	—	57
19	52	2	—	—	—	—	—	54
20	40	3	—	—	—	—	—	43
21	19	6	—	—	—	—	—	25
22	15	8	1	—	—	—	—	24
23	3	10	—	—	—	—	—	13
24	2	13	3	—	—	—	—	18
25	1	20	3	—	—	—	—	24
26	2	18	4	—	—	—	—	24
27	1	18	13	—	—	—	—	32
28	—	9	6	1	—	—	—	16
29	—	6	8	2	—	—	—	16
30	—	3	5	2	—	—	—	10
31	—	4	4	—	—	—	—	8
32	—	—	2	1	—	—	—	3
33	—	2	5	1	—	—	—	8
34	—	—	1	—	—	—	—	1
35	—	—	2	—	—	—	—	2
36	—	—	—	—	—	—	—	—
37	—	—	1	1	—	—	—	2
38	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—
41	—	—	—	1	1	—	—	2
42	—	—	—	—	—	—	—	—
43	—	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—
47	—	—	—	—	—	—	—	—
48	—	—	—	—	—	1	—	1
49	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—
Total	331	125	58	9	1	1	—	525
Mean length	18·53	25·71	29·26	32·6	41	48	—	
Mean length 1950+1951	18·86	25·35	30·0	35·2	41·1	46·3	49·75	

J. Mar. biol. Ass. U.K. (1954) 33, 123–127
Printed in Great Britain

NOTES ON THE EARLY STAGES OF THE COMMENSAL POLYNOID *ACHOLOË ASTERICOLA* (DELLE CHIAJE)

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(Text-fig. 1)

Little is known about the development of polynoids. A large number of unidentifiable polynoid trochophores and nectochaetes have been described by Nolte (1936) and others. As Thorson (1946) states, Nolte has done little to clear up the confusion that prevails in this group and his 'numerous figures, all more or less incorrect, and accordingly difficult to recognize, merely serve to further obscure our picture'.

A search of the literature with the aid of Hartman (1951) has so far revealed only one clearly identifiable member of the family whose early larval stages have been described. Sars (1845*a*, *b*), making some of the earliest observations on polychaete life histories, described under the name *Polynoë cirrata* Fab. the early stages of *Harmothoë imbricata* (L.). Further studies in some detail on this or a closely allied species were made in turn by Müller (1851), McIntosh (1900) and Izuka (1912). The certainty of identification of the parent in this case stemmed from the fact that the early stages, with the exception of those studied by Müller, were not taken in the plankton but were observed attached to the brooding parent after release from it. In this polynoid the eggs are carried in large masses beneath the elytra, and larvae leave the parent as actively swimming trochophores. This method of carrying the young has also been observed in *H. imbricata* by Saemundsson (1918) in Greenland and by M. Pettibone (personal communication) in Puget Sound. Desor (1857) quotes the observations of Sars on the brooding habit, and describes the release of trochophores by *Polynoë squamata* (= *Lepidonotus squamatus* L. ?) on the New England coast. He does not make clear whether the form he studied also brooded its eggs beneath the elytra. However, Fauvel (1916) observed brooding in *Polynoë antarctica* Kinberg, from the Falklands, and MacGinitie & MacGinitie (1949) in *Halosydna brevisetosa* Kinberg from the eastern Pacific. Thus, brooding in polynoids may be commoner than has been suspected.

From October of 1952 until July of 1953, while the author held a Guggenheim Fellowship at the Plymouth Laboratory and was engaged in making studies (Davenport, 1953*a*, *b*) of the response behaviour of commensal annelids to their hosts, repeated efforts were made to bring to metamorphosis and settlement the young of the polynoid *Acholoë astericola* (Delle Chiaje), commensal with the starfish *Astropecten irregularis* (Pennant). Although in a number of cultures it was possible to carry the larvae through 10–12 days of development, none were taken beyond the trochophore stage; critical experiments to determine the manner of settlement and the method of host-colonization were never possible. In view, however, of the general lack of information concerning polynoid development and the certainty of identification of the young of only one other polynoid species at the present time, it has seemed advisable to describe the stages observed, in spite of failure in bringing the life history to completion.

At no time from October to July was natural spawning observed, in spite of the fact that *Acholoë* were brought into the laboratory on their host starfish continuously during this time and kept under a variety of conditions. Eggs and sperms were obtained by cutting open worms, and fertilizations were made in filtered 'outside' sea water. Dishes were kept at the temperatures of the laboratory circulation (14–16° C.). Cultures were also placed in large plunger jars. Although trochophores were observed to feed on pure cultures of the flagellates *Dicrateria inornata* Parke and *Isochrysis galbana* Parke, after an average of 10 days all dropped to the bottom, became inactive and broke up.

The sequence of events to be described was observed with fertilizations made in the month of November, at which time in any collection the number of adult worms with active sperms or fully formed eggs was low. During the winter the number of such individuals in any collection approached nil. During the months of April and May apparent fertility increased until in July almost all individuals opened had active sperms or fully formed eggs. Thus, it would appear that *Acholoë* is a midsummer or autumn spawner, at least at the latitude of Plymouth.

Eggs freshly removed from the body cavity were pinkish in reflected light and slightly oval, the long dimension averaging approximately $85\,\mu$, with a membrane approximately $1\cdot5\,\mu$ in thickness. Very shortly after fertilization the membrane separated from the eggs and there often appeared a slightly raised area or 'bubble' at one pole. From 12 to 14 hr. after fertilization larvae were actively spinning and the beginning of ciliary bands had appeared. The long dimension had by this time increased to about $97\,\mu$.

By the end of 48 hr. many of the oval prototrochophores (Fig. 1A) were actively swimming, rotating on their long axes and moving in the direction of the apical tuft. Their long dimension at this time was approximately $100\,\mu$. The apical tuft was composed of fifteen or more very long cilia fully two-thirds the length of the larva ($65\,\mu$), while the clearly evident prototroch consisted of a single band of cilia which were approximately $20\,\mu$ long. An opaque central area indicated the beginning of the digestive tract.

By the end of 72 hr. the larvae had considerably advanced, although there was little change in size. Most had developed a single red eye-spot, though in some two had appeared. There was some reduction in size of the apical tuft, still a single-rowed prototroch and as yet no other groups of cilia. Rather large yellowish green pigment bodies had begun to make their appearance in the apical and prototrochal region and in the hyposphere.

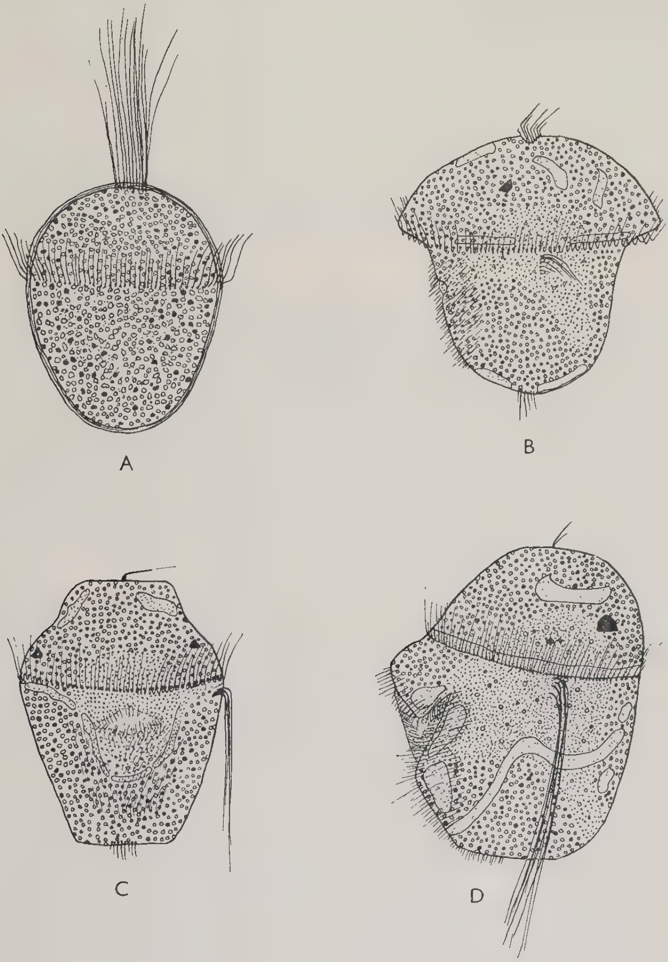

Fig. 1. Early stages of *Acholoë astericola* (Delle Chiaje). A, prototrochophore at 48 hr.; B, trochophore at 168 hr., showing beginning of left lateral tuft; C, trochophore at 216 hr. in ventral view; D, 9-day trochophore of July culture in left lateral view.

By the fourth day one could discern stomach and intestine, in which there was ciliary activity. A small clump of cilia marked the beginnings of the telotroch. Most individuals possessed two eye-spots. At this time the larvae had begun to assume the typical shape of the early trochophore in which the episphere appears to overhang the hyposphere.

By the sixth day little change had occurred, but one could discern a definite ventral ciliary band or neurotroch.

Fig. 1B shows a trochophore of this culture at 168 hr. in lateral view. There had been little increase in length. The cilia of the apical tuft were reduced in number and length, there being eight or nine cilia some 20 μ long sunk in a small pit. The neurotroch widely covered the indentation of the mouth and extended over a quarter of the circumference of the hyposphere. The telotroch consisted of a few short cilia. At this stage there began to be apparent a short, comb-like, scimitar-shaped lateral tuft of cilia on the left side of the trochophore.

Fig. 1C shows a larva of this culture in ventral view at 216 hr. A slight increase in size of the long dimension (107 μ) was accompanied by a relative reduction of size of the episphere relative to the hyposphere. The mouth was extensive and clearly discernible, while the greenish yellow pigment bodies were marked. The apical tuft was reduced to a very short, fused unit. The left lateral tuft, hanging down from the 'belt' of the prototrochal region like a scabbard, had increased greatly in length until it was fully two-thirds the length of the larva, some 63 μ. This structure is one of the most marked diagnostic characters of the *Acholoë* trochophore; such a structure has also been described in the trochophore of *Nephthys* by Wilson (1936).

Fig. 1D shows a 9-day larva of a July culture in left lateral view. The trochophores of this summer culture averaged some 130 μ in length, although they showed no structural differences from larvae raised in the fall. The extent of the oral lappet and the depth of the pit are evident. Such larvae, the most advanced to be observed, demonstrated considerable muscular activity. All, in spite of feeding and apparent health, failed to develop further.

In the various cultures studied the steps in development were similar, but in mid-summer cultures the whole process, perhaps because of higher temperatures or perhaps because the eggs were more nearly ripe when fertilized, took place in a shorter time, so that larvae took no more than 3 days to reach the stage which was reached by late fall larvae in not less than 7–9 days.

From the above studies it would appear that *Acholoë* has an early development that allows the organism to be widely dispersed by ocean currents, and in this respect, in spite of its being an obligate commensal as far as is known, is similar to other polynoids. The problems of settlement and 'colonization' of host remain unsolved. No young worms smaller than 0·5 cm. have ever been observed on *Astropecten*. Do the larvae metamorphose on the correct substrate and later find the host? It is possible that if eggs and sperm can be had from naturally spawning adults, and if problems of temperature and feeding can be met, such questions may ultimately be answered.

SUMMARY

The development of the larva of *Acholoë astericola* (Delle Chiaje), a commensal of *Astropecten irregularis*, is described up to the late trochophore stage. The larva of only one other identifiable polynoid worm had previously been described.

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J. Mar. biol. Ass. U.K. (1954) 33, 129–144
Printed in Great Britain

THE ANATOMY OF THE PROSOBRANCH *TRICHOTROPIS BOREALIS* BRODERIP & SOWERBY, AND THE SYSTEMATIC POSITION OF THE CAPULIDAE

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(Text-figs. 1–4)

The anatomy of some members of the family Calyptraeidae has been adequately described by previous workers (Kleinsteuber, 1913; Giese, 1915; Moritz, 1938), and a considerable amount of information has been published on the feeding and the unusual reproductive activities of *Crepidula* (Orton, 1912; Coe, 1944). Of the anatomy and way of living of the members of those other families of gastropods (Trichotropidae and Capulidae) that Thiele (1929) has united with the calyptraeids in his Stirps Calyptraeacea, much less is known. Orton (1912) and Yonge (1938) have described the feeding mechanism of *Capulus ungaricus*; and the anatomy of *Thyca*, a parasitic member of the same family, has been briefly described by Koehler & Vaney (1912). Nothing seems to be known of the anatomy or way of life of members of the Trichotropidae. Yet an investigation of some of these points seems overdue in the light of Lebour's discovery (1937) that *Capulus* passes through an echinospira larval stage. This stage does not occur in other members of the Calyptraeacea, although present in two other groups of the mesogastropods, the Lamellariacea and the Cypraeacea. The idea at once presents itself that the capulids may perhaps be more accurately classified as members of one or other of these two groups rather than as members of the Calyptraeacea. The work recorded in the following pages was directed towards answering this question, and would suggest that the original classification is the more correct.

The names used are those of Winckworth (1932).

Anatomical observations

Trichotropis borealis Broderip & Sowerby

The family Trichotropidae, of which *Trichotropis borealis* is the sole British representative, is on the whole confined to colder seas. In Britain *T. borealis* is restricted to the northernmost parts of England and to Scotland, and is not littoral. The animals used in this work were collected off Oban by Dr V. Fretter of Birkbeck College, University of London, and it is to her kindness and to that of Mr T. G. W. Fowler, on whose boat she was working, that I owe the opportunity of examining them. They were found at a depth of 2–3 fathoms on clinker cast overboard from the fire grates of the West Highland boats. The material was fixed in Bouin and proved very difficult to section: I am indebted to Mr C. Best of the Otological Research Unit of the Medical Research Council at the National Hospital for Nervous Diseases, London, for a magnificent series of celloidin sections cut 10 μ thick.

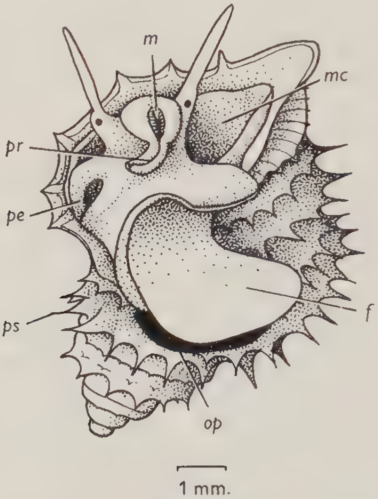

Fig. 1. *Trichotropis borealis*, drawn from a living specimen. *f*, foot; *m*, mouth; *mc*, opening to mantle cavity; *op*, operculum; *pe*, penis recurving into mantle cavity; *pr*, proboscis curving from mouth to right side of foot; *ps*, periostracal spines on shell.

In respect of its external features *T. borealis* presents the usual appearance of a small prosobranch with spirally coiled shell (Fig. 1). The shell itself is well covered with thick periostracum which is drawn into numerous pointed processes (*ps*) set especially along the lines of the spiral ridges running round the whorls. When the head and the foot are extended, it may be seen that the tentacles are long and slender, with the eyes placed about one-quarter of their length from the base. The foot (*f*) is rather abruptly cut off both in front and in the rear with a slight narrowing in the middle, but it is mobile and there does not appear to be any specialization of its anterior end, except for a degree of elongation. The posterior end carries a horny operculum (*op*). The opening into the mantle cavity (*mc*) is large and the edge of the mantle skirt is plain and does not appear to contain marginal glands apart from those concerned with the secretion of the shell. The only other point in the external characteristics of the mollusc is the presence of a short proboscis extending forwards from the mouth (*pr*). Like that of *Capulus*, this proboscis is grooved on the upper side to produce a channel which runs from the tip of the proboscis along its length to the mouth (*m*) at its base. In normal circumstances the proboscis is kept with its tip turned towards the animal's right so that it lies dorsal to the front end of the foot and rests along the right side of that organ. A similar curvature was noted in respect of the proboscis of *Capulus* by Orton (1912) but denied by Yonge (1938). The surface of the proboscis is not ciliated except on the floor of the groove, and the structure in *Trichotropis* seems to function in the same way as the corresponding organ in *Capulus*. When the process of feeding is investigated by means of carmine powder suspended in sea water it is found that a strong current enters the mantle cavity on the animal's left, that particles from this fall on to and travel across the floor of the mantle cavity and are then carried along the right side of the head and foot, as in *Capulus*. In this way they reach the tip of the proboscis, travel up its groove and at the base of this are licked into the buccal cavity by means of the radula.

Small specimens of *Trichotropis* possess a penis (*pe*), a slightly flattened finger-shaped structure grooved on the lateral side and normally lying recurved in the mantle cavity. It is a solid organ without detectable blood spaces and there are no glands on its surface. From its base the seminal groove may be traced to the genital aperture, an elongated slit lying on the right side of the mantle skirt. Larger specimens lack the penis and are female, so that the animals seem to be successive hermaphrodites and protandrous.

The mouth leads to the buccal cavity, within which lies the buccal mass carrying the radula and strengthened by a pair of cartilages, and into which a pair of salivary glands discharges by very short ducts. The glands are small lobed pouches and manufacture a secretion of almost pure mucus, the epithelium lining the walls being composed of mucous cells alternating with slender supporting cells which are sometimes ciliated. The surface of the buccal cavity is covered with cuticle in most areas, but there is no well developed jaw. The oesophagus is long, running back to the stomach in the visceral hump. The glands of the mid-oesophagus are well developed and the morphologically ventral part of this section of the oesophagus has walls flung into a series of obliquely directed lamellae. The longitudinal dorsal folds, which separate the dorsal food channel from the glands, are long, project deeply into the lumen of the oesophagus and are wrapped one over the other so as to isolate the two parts of the oesophagus more or less completely from each other. The posterior oesophagus is, as is usual in prosobranchs, a simple tube with a ciliated columnar epithelium containing mucous glands.

It leads to the stomach, a not very capacious cavity lying directly underneath the mantle on the outer side of the visceral hump. The oesophageal opening and that of the style sac lie almost side by side at its lower end, and two wide ducts from the digestive gland open to the stomach close to the same point. From one of these the major typhlosole emerges and runs along the style sac; the minor typhlosole, which in the style sac forms the other boundary of the intestinal groove, dies away on the stomach wall between the two apertures of the digestive gland. A considerable area (perhaps most) of the rest of the stomach wall is covered with cuticle which is raised slightly at one point to form the gastric shield. The epithelium which lines the stomach is tall and columnar, either ciliated or bearing the cuticle; apart from this it is rather featureless. In the style sac there appears the characteristic ciliated epithelium of this part of the molluscan stomach—low columnar cells, with homogeneous cytoplasm and large nuclei, bearing many cilia—with taller, narrower cells and mucous cells in the typhlosoles. No crystalline style is apparent, nor, in view of the existence of well-developed oesophageal glands, is it likely that one exists (Graham, 1939).

The digestive gland occupies the outer half of the more apical whorls of the visceral hump. Its tubules contain two types of cells, one much more abundant than the other, and clearly responsible for most of the digestive activity of the gland. I am not certain what is the function of the other type, since I have not had enough animals to allow observation in different physiological states or to permit experiment. The first type, the digestive cell (Fig. 2, *dc*), is a tall club-shaped cell with an inconspicuous nucleus lodged in the narrow base and swelling to a tumid apex bulging into the cavity of the tubule. There are clear signs in the material that this type of cell takes particles of food into food vacuoles for an intracellular digestive process, and I suspect that the distal end of the cell is nipped off with zymogen granules at another stage of the cycle of activity.

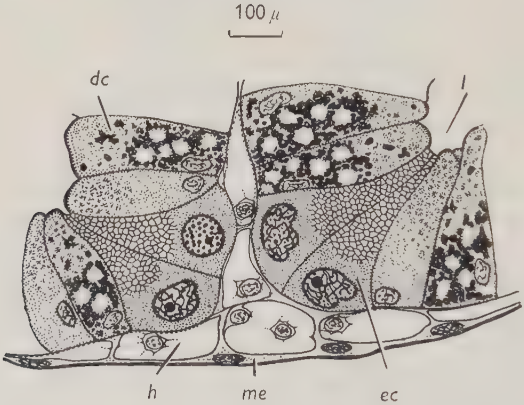

Fig. 2. *Trichotropis borealis*. Part of section across digestive gland. *dc*, digestive cell; *ec*, excretory cell; *h*, interlobular haemocoelic space; *l*, lumen of tubule; *me*, epithelium of mantle.

The second type of cell (*ec*) is much less abundant and is located in crypts in the tubules especially on the sides which face the mantle (*me*). Its shape is more or less triangular in section with the base broad and a narrow apex reaching to the lumen of the tubule (*l*). The cytoplasm is not vacuolated, except slightly so near the apex; at the base it is dense and stains rather darkly with iron haematoxylin. The outstanding feature of the cell is the nucleus, which is large, lobed, and contains a prominent nucleolus and numerous granules of chromatin. All these features suggest a high level of metabolic activity, and the broad base which the cell presents to the haemocoelic spaces (*h*) between neighbouring tubules of the gland would indicate that the cell is responsible for some process which involves the transfer of material from blood to cell or vice versa. In other gastropods (see below) this type of cell is often concerned with excretion, and this may be true in *Trichotropis* too, but I have insufficient evidence on the point.

The intestine starts at the distal end of the style sac and runs across the body from left to right to open at the anus, which is situated a little way in from the mouth of the mantle cavity. The whole length is ciliated and glandular with a thin layer of muscle external to the epithelium. Near the stomach the wall is particularly rich in mucous cells and these again become abundant near the anus; in the intervening length of intestine, however, mucous cells are scarce and the glands appear to be manufacturing some type of protein secretion since they stain intensely with such stains as iron haematoxylin. The lips of the anus, which is placed on a short papilla, are drawn out into a filament of some length hanging freely into the pallial cavity.

The faecal pellets are well compacted, short, oval bodies containing sand grains, diatom cases, sponge spicules and vegetable debris—the typical faecal contents of a detritus feeder.

In the nervous system of *Trichotropis borealis* the cerebral ganglia lie close together above the oesophagus and posterior to the salivary glands. Behind them and below are the pleural ganglia, so closely associated with the cerebrals as to form a single dumbbell-shaped mass of nervous tissue on each side. The pedal ganglia with their associated statocysts, however, lie well forward and ventrally so that the cerebropedal and pleuropedal connectives are relatively lengthy. The supra-oesophageal and suboesophageal ganglia lie close to the oesophagus, the former a little posterior to the nerve ring, the latter directly under the right pleural ganglion. It has, in fact, established a zygoneurous connexion with this which is absent on the other side of the body. No dialyneury exists on either side. The visceral ganglia lie ventrally in the visceral hump, both to the left side of the mid-line, one close to the kidney, which it innervates along with the reproductive apparatus, the other near the pericardial wall, to which it sends branches as well as supplying the stomach and the digestive gland.

The osphradium, ctenidium and hypobranchial gland are all well developed structures within the mantle cavity. The osphradium is a bipectinate structure as in many prosobranchs. The gill lies along, rather than across, the mantle cavity, and the filaments are like those of *Capulus* as described by Yonge (1938). The hypobranchial gland is extensive and rich in secreting cells, of which there appear to be three different sorts, intermingled with ciliated cells. The last are excessively slender cells with wedge-shaped distal ends squashed between the bulging gland cells. Most of the gland cells secrete mucus, but there are also two kinds secreting material which is not of that nature. Mucous cells also occur on the gill filaments and abound on the floor of the mantle cavity so that vast quantities of that substance are available for the trapping of food particles and for their transport to the proboscis. The mantle skirt and the internal parts of the body are largely occupied by a coarsely reticulate connective tissue.

The gonad, a testis in young animals and an ovary in older ones, occupies half of the upper whorls of the visceral hump, lying on the concave inner side of the spiral. It is divided into lobes which converge on a gonadial duct which takes a convoluted path down the visceral hump to the renal and pallial sections of the reproductive tract (Fretter, 1946). In the male phase this section is used as a seminal vesicle and is occupied by masses of mature sperm, unorientated. It is not muscular and is lined by a squamous epithelium which is not ciliated.

Near the ventral end of the visceral hump the gonadial duct joins the renal section of the genital tract, a narrow tube with a ciliated cubical epithelium and some muscle fibres in the walls. It has no pericardial connexion. The renal vas deferens ends by opening into the pallial section, which, even in the male phase, is already showing the rudiments of the female accessory glands, and which may, perhaps, have some prostatic function. The male duct leads on to a ciliated tract which runs along the right (outer) wall of this section. The duct opens to the mantle cavity by a narrow, slit-like aperture and the ciliated tract extends as far as the anterior border of this. For a short distance before it reaches this point, however, it is applied against a similar ciliated tract located on the right body-wall of the snail. Sperm can therefore pass, partly by ciliary action, partly by their own movement, from the genital duct to the seminal groove and so to the penis.

Even in the male phase, as was noted by Giese (1915) in the course of his investigation of the genital tracts of the calyptraeids and of *Capulus*, the beginnings of the later female system are well developed. In *Trichotropis* a receptaculum seminis runs dorsally from the point where the renal and pallial sections of the duct join, in the form of a narrow, blind tube, the inner end of which branches to form a tuft of three short tubules. The main part of the pallial section takes the form of a large glandular pouch within which areas may be distinguished as the albumen and capsule glands. The fundamental histological structure of all these regions is the same—excessively tall and narrow supporting cells, often ciliated, being wedged between gland cells, all of which lie within the basement membrane of the epithelium.

Variation affects only the nature of the secretion elaborated by the glands. In the albumen gland the cells contain spherules which do not stain with iron haematoxylin; while in the capsule gland the granules of secretion usually do take up this stain and there are also, at its inner and outer limits, strips of cells which are exclusively mucous. There is no visible bursa copulatrix, and on copulation it must happen that the penis is inserted directly into the capsule gland.

The oldest animal of which I had sections had still ripe spermatozoa in the spaces of the gonad and in the proximal section of the genital tract, whilst the receptaculum seminis was empty: the animal was therefore still functionally male. Nevertheless, the gonad contained eggs of all sizes, some of which measured 0·3 mm. in diameter and were laden with yolk granules. It appears that this animal was close to the point when change from male to female was imminent, and it would probably have acted as female at the next reproductive phase.

Capulus ungaricus (L.)

The feeding mechanism of this animal has been described by Orton (1912) and Yonge (1938), and the contents of the mantle cavity noted, but otherwise its anatomy is not well known. *Capulus* is limpet-like in shape, with the shell tip tilted backwards like a Phrygian cap. The shell has a wide and round mouth which cannot be closed by an operculum, and it is covered by a thick, brown, periostracal layer, fringed at the edges but smooth elsewhere. The head is not elongated, bears two short, flattened tentacles with eyes at their bases and also a proboscis like that of *Trichotropis* but longer, with the mouth at its base and a groove dorsally. As Yonge (1938) notes, it is mobile and not restricted to one position as that of *Trichotropis* appears to be. The foot is rounded but has a thin anterior part which can be stretched well forward, and it is on to the upper surface of this, as Yonge (1938) has shown, that the food particles from the mantle cavity are led before being licked up by the proboscis. The opening into the mantle cavity is large and the edge of the mantle skirt is simple and devoid of marginal glands apart from the shell gland. The columellar muscle is horseshoe-shaped with the mantle cavity embraced between its two anteriorly directed arms. In this the lobed osphradium and the ctenidium extend obliquely forward from the left arm of the horseshoe, with the space between ctenidium and the right arm of the horseshoe occupied by the hypobranchial gland, the rectum and the reproductive ducts.

The alimentary tract of *Capulus* is simpler than that of *Trichotropis* but in general is similar to it. The oesophageal region, as noted earlier (Graham, 1939), is devoid of glandular equipment apart from mucous cells, and is a relatively simple tube passing to the stomach. The stomach (Fig. 3) lies in a vertical position on the left side of the visceral hump, its morphologically anterior end almost at the apex of the hump, where the oesophageal aperture is placed, whilst the intestine opens from the style sac ventrally. As in *Trichotropis*, two large ducts lead from the digestive gland, both opening near the point of entry of the oesophagus. From one of these the major typhlosole (t_1) runs to the style sac, the minor typhlosole (t_2) originating on the stomach wall near the oesophageal opening. A gastric shield (*gs*) lies on the right side of the stomach. From the stomach the intestine (*i*) runs across to the anus and is flung into a posteriorly directed loop on the way. It is rich in mucous cells, especially near the anus.

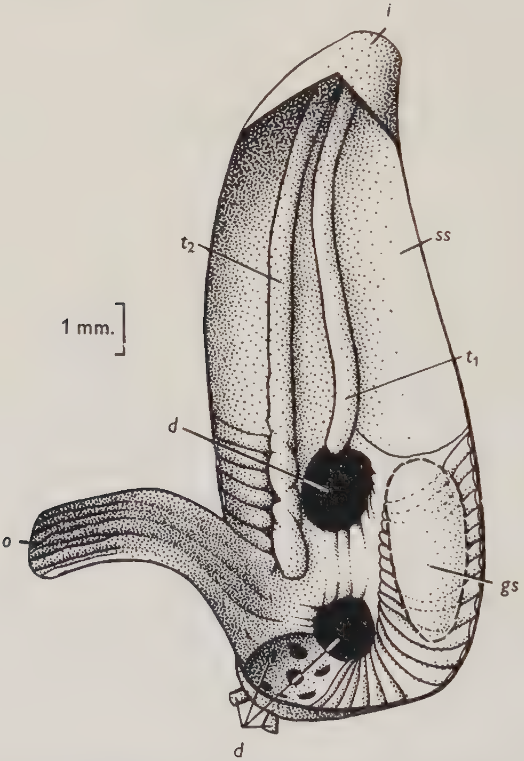

Fig. 3. *Capulus ungaricus*. Dissection of stomach. *d*, ducts of digestive gland; *gs*, gastric shield; *i*, intestine; *o*, oesophagus; *ss*, style sac; t_1, major typhlosole; t_2, minor typhlosole.

In the digestive gland of *Capulus*, as in that of *Trichotropis*, two cell types occur: one digestive, the second with another function. The former is a tall club-shaped cell presenting indications of secretory or phagocytic activity, depending upon the stage in the digestive cycle at which the mollusc has been killed. The second cell is lower, of pyramidal shape, and is tucked into the angles of the tubule with the broad base presented to the intertubular haemocoelic spaces. The cell has dense cytoplasm with a few granules embedded in it and a large lobed nucleus. It is difficult, in the absence of experimental results, to be any surer of the action of this type of cell in *Capulus* than of its homologue in *Trichotropis*; but the activity is more likely to be secretory or excretory than concerned with the direct manipulation of food particles.

Capulus possesses a nervous system (Fig. 4*A*) built upon similar lines to that of *Trichotropis*, the sole major difference detected between the two being that in addition to the zygoneurous connexion established between the right pleural (*pg*) and the suboesophageal (*sbg*) ganglia, there is a dialyneury (*d*) brought into being by an intermingling of the left pallial nerve (*lpn*) from the left pleural ganglion with the osphradial nerve (*lon*) from the supra-oesophageal ganglion (*spg*): this occurs where the two nerves pass alongside one another over the shoulder of the columellar muscle on their way into the mantle skirt. The two visceral ganglia (*vg*) lie ventrally in the basal part of the visceral hump, as in *Trichotropis*, though perhaps more closely than in that mollusc.

As is well known, *Capulus* is a successive hermaphrodite, each animal passing through a young male phase, when it is provided with a penis and the rudiments of the accessory female glands, to reach a definitive female phase when the penis is lost. The general anatomy of the reproductive system has been previously described by Giese (1915), and I have nothing significant to add to his account of this system: its most important features are the absence of a gonopericardial duct, the presence of a seminal groove from genital pore to penial apex, the lack of penial glands in the male phase, and the development of an albumen gland and receptaculum seminis at the inner end of the capsule gland in the female stage. The receptaculum has the form of a finger-shaped pouch slightly branched at the distal extremity. There is no bursa copulatrix.

Calyptraea sinensis L. and *Crepidula fornicata* L.

The anatomy of these animals agrees in all points with that of the calyptraeids described by Kleinsteuber (1913) and Moritz (1938). It need not, therefore, be elaborated; and it will suffice to mention points of difference between the members of this family, the trichotropids and the capulids. While the general body shape is limpet-like, more of the spiral coiling of the visceral hump has been retained in the calyptraeids than in *Capulus*, although the visceral mass is completely symmetrical in the related genus *Janacus* (Kleinsteuber, 1913). The mantle edge, though still smooth, shows more complexity than in the other families: it is bifurcated and folded over the entrance to the mantle cavity so as to form the food pouch described by Orton (1912) and by Yonge (1938); it is also more extensive, forming a large shelf-like projection round the body of the gastropod. It is no longer simple, but shows a triple folding, the most dorsal part being a simple fold in direct contact with the shell above, lodging the shell gland and covered by an unciliated cubical epithelium ventrally. The middle fold, by far the largest of the three, contains blood vessels, two circumpallial nerves and a series of large marginal glands which discharge to the ventral side. This fold of the mantle edge has retractor muscle fibres running into it which originate on the shell, and it is covered laterally and medially by an unciliated cubical epithelium, but on its ventral surface by a ciliated columnar epithelium which is rich in mucous cells. The marginal glands are spherical bodies opening by a long, narrow duct lined by squamous cells. The secreting cells are grouped to form the spherical body and are filled with dense cytoplasm. The nature of the secretion is unknown, but it seems to be secreted on noxious stimulation and the glands are therefore repugnatorial. The third fold of the mantle edge is a small one constricted off the medial edge of the second. This triple subdivision of the pallial edge is, as already noted by Odhner (1932), characteristic of the archaeogastropod and bivalve mantle and would seem to be primitive.

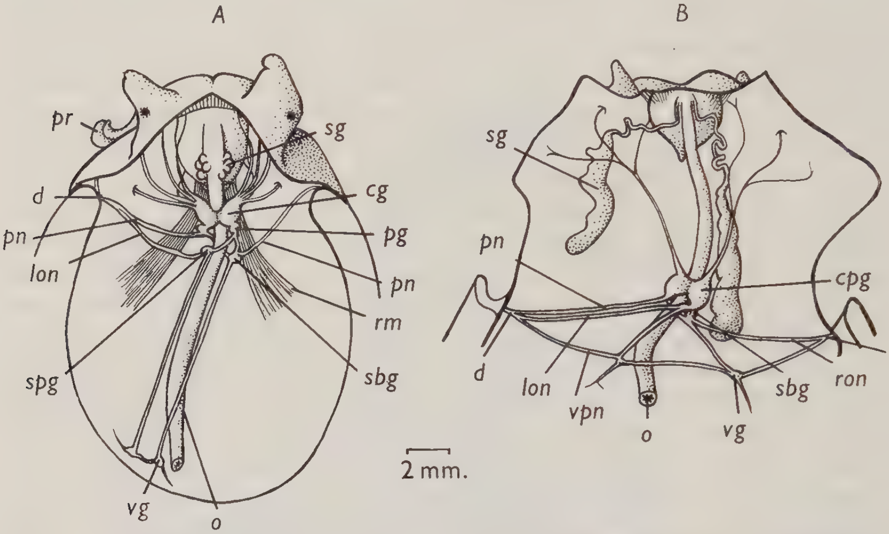

Fig. 4. *A*, dissection to show part of the nervous system of *Capulus ungaricus*. *B*, dissection to show part of the nervous system of *Crepidula fornicata*. *cg*, cerebral ganglion; *cpg*, fused cerebral and pleural ganglia; *d*, point of dialyneury; *lon*, left osphradial nerve from supra-oesophageal ganglion; *o*, oesophagus; *pg*, pleural ganglion; *pn*, pallial nerve from pleural ganglion; *pr*, proboscis; *rm*, retractor muscle of proboscis; *ron*, right osphradial nerve from suboesophageal ganglion; *sbg*, suboesophageal ganglion; *sg*, salivary glands; *spg*, supra-oesophageal ganglion; *vg*, visceral ganglion; *vpn*, pallial nerve from visceral ganglion.

The head of a calyptraeid has been elongated in what may be spoken of as the 'neck' region, that is behind the tentacles and penis, and is there provided with a pair of lateral extensions, the cephalic lappets. Their development is associated with the ciliary food-collecting mechanism of these animals. In relation to that, too, the mantle cavity extends far up the visceral hump on the left side, forcing the shell muscles to lie on the right and preventing them from forming a horseshoe as in *Capulus*. The length of the gill filaments, as described by Orton (1912) and Yonge (1938), has also increased in this connexion and the ctenidial axis (Graham, 1939) has been converted into what Orton (1913) described as an 'endostyle'—an accessory gland producing mucus for the trapping of the food particles. The osphradium is small and pectinate; the hypobranchial gland is well developed.

The alimentary canal (Graham, 1939) is simple. In the buccal cavity the radula is weak and rests on a small buccal mass. The salivary glands (Fig. 4*B*, *sg*) are larger structures than in either trichotropids or capulids. They are tubular, reaching posteriorly from the buccal cavity as far as the level of the nerve ring, the anterior third of this length being occupied by a narrow duct, the rest by gland. In the glandular section three types of cell occur: narrow, ciliated cells squashed between gland cells of two sorts, the more numerous secreting a substance which is not mucus, the second type secreting mucus. There are no oesophageal glands and the stomach contains a crystalline style. The oesophagus enters it at its posterior end and the intestine, which leaves the style sac anteriorly, has a figure-of-eight loop on its course to the anus. The digestive gland contains two types of cell, one digestive and the other excretory. This second type invariably has large vacuoles in the cytoplasm in which lie concretions, often yellow in colour and partly crystalline in nature, though in other individuals the vacuoles may be filled with many minute granules. Since these concretions may also be seen in the faeces, and since the cells in which they lie have broad bases facing the blood spaces in and around the digestive gland, it seems safe to suggest that they consist of waste material. Nothing comparable may be detected in the other cells of the digestive gland, although it is into these that experimentally introduced foods find their way. The excretory matter in the other cells appears therefore to be endogenous rather than faecal in nature. It is possible that no secretion of digestive enzymes takes place from the gland.

The excretory organ is clearly composed of a nephridial gland in close relationship to the pericardial cavity and a large nephridial sac discharging to the mantle cavity.

The nerve ring lies at the posterior end of the 'neck' and therefore some way behind the buccal mass. The dorsal ganglia—an apparently single pair—are formed from fusion of the cerebrals and pleurals, the boundaries between the two being obliterated (Fig. 4*B*, *cpg*). On the right side the supra-oesophageal ganglion lies contiguous to the right pleural, of which it simply appears to be a lobe, whilst the suboesophageal (*sbg*) lies similarly ventrally. Still more ventrally are placed the pedal ganglia. A zygoneury has therefore been established on the right side, and since fusion occurs on the left (near the point where the nerves pass to the mantle skirt) between the osphradial nerve from the supra-oesophageal ganglion (*lon*), the pallial nerve from the left pleural ganglion (*pn*) and a pallial nerve from the visceral (*vpn*) and, similarly, on the right between osphradial nerves from the suboesophageal ganglion (*ron*) and a pallial branch from the right visceral, there is a very complete link up in the nervous system. The two visceral ganglia (*vg*) lie only a short distance posterior to the nerve ring and also rather far apart, so that the visceral loop has a triangular shape with the base between the two visceral ganglia.

The general plan of the reproductive system of calyptraeids has been described by Giese (1915), and I have nothing to add. The animals are protandrous hermaphrodites, the duct has a gonopericardial canal and the penis has glands at its base and apex.

Discussion

When the anatomical points described above are taken into consideration, it seems that all three families of gastropods may fairly be thought to possess a high degree of similarity of structure.

The shell in at least two of the three families shows a thick periostracal layer which tends to be drawn out into processes. In accord with their mobility the trichotropids preserve the spirally wound visceral hump and shell of the typical prosobranch. They may gather food with their proboscis as well as collect it out of the water current maintained through the mantle cavity. With greater dependence on this current as a source of food less mobility is required and the limpet-like shape of *Capulus* and the calyptraeids is adopted, although that has been achieved in different ways in these two groups, the mantle cavity being elongated in a transverse direction in the capulids (which allows the formation of a horseshoe-shaped columellar muscle) and in a longitudinal direction in the calyptraeids. The evolution of repugnatorial glands is presumably related to the lack of operculum and the immobility of the animals. A similar evolution attends the alimentary tract: *Trichotropis* has an oesophagus which shows the usual prosobranch structure and the animal has no crystalline style. In the calyptraeids there has been developed the style, presumably as a ready source of enzyme in connexion with the microphagous habit; and it seems likely that *Capulus* has evolved in the same way. The oesophageal glands, as is usual in such cases, are lost and the radular apparatus reduced, though there is increasing complexity in the mantle cavity as that part of the body evolves in efficiency as a food collecting apparatus. The fundamental organization of the stomach, with intestinal and oesophageal apertures at opposite ends, two large openings to the digestive glands close to the oesophageal aperture and no spiral caecum, appears similar in all, as does the structure of the gland itself with two contrasting types of cell. In each of the types the intestine runs across from left to right to the anus and is flung into a backwardly directed loop on the way.

In all three groups the nervous system is modified in identical fashion and the only significant difference is in the varying shape of the visceral loop: this is long in *Trichotropis*, moderately long in *Capulus* and distinctly short and triangular in the calyptraeids. But that is, in fact, the kind of change in shape which would accompany a change from the normal prosobranch appearance to the limpet facies and probably has no deeper significance.

The reproductive system is a further example of agreement. All the animals are hermaphrodite; all have the rudiments of the female organs present in the young, male, phase; and all have a seminal groove leading forwards to and along a slender penis which disappears when the animal becomes female. The plan of the glands in the female phase is similar in all three. It seems to be only in *Crepidula* that this special timing of reproductive activity has become the basis of special behavioural patterns.

In their reproduction, too, there seems to be a certain degree of similarity. All appear to lay their eggs in capsules (Thorson, 1935; Lebour, 1937) which may be fastened to bivalve shells (*Trichotropis*), or to stones or shells; they are often incubated by the parent (*Calyptraea*, *Crepidula*, *Capulus*). All, however, differ in the stage at which the young animals escape. In *Crepidula* the eggs give rise to free-swimming veliger larvae; in *Capulus* to free-swimming echinospira larvae; whereas in both *Calyptraea* and *Trichotropis* the veliger is suppressed and the young hatch as crawling miniatures of their parents.

The echinospira larva occurs also in Lamellariacea and in Cypraeacea. To what extent do the members of these groups resemble the Calyptraeacea anatomically? Their anatomical characters have been described by Vayssière (1923), Thiele (1929), Rau (1934) and Fretter (1946, 1951) and may be summarized in the following paragraphs.

There is little tendency for the production of periostracum around the shells of these animals; instead, another tendency becomes obvious—for the mantle to grow over the shell and ultimately enclose it completely. The mantle edge has no special marginal glands but is extended into a respiratory siphon.

The animals are all macrophagous carnivores, provided with jaws and proboscis for the manipulation of their prey, which tends to be predominantly sessile tunicates and coelenterates—as must, inevitably, be the case when such a slow-moving animal as a gastropod mollusc turns carnivore (Graham, 1953). The alimentary canal is well provided with extensive oesophageal glands (Amaudrut, 1898; Fretter, 1951) and the stomach is on the whole simpler than that of the calyptraeacean, as might be expected in view of their food (Graham, 1949).

The foot (Fretter, 1951) is provided with a posterior mucous gland in addition to the ordinary anterior one and in that respect differs from the foot of the calyptraeacean, although there is a tendency in all three groups to lose the operculum. The explanation of the loss is, however, clearly different in the two cases: in the one it is part of the trend towards a limpet-like way of living; in the others it is part of the trend towards an internal shell.

Other general resemblances between the two groups of gastropods become obvious when their nervous and reproductive systems are compared. In the former there is a considerable degree of concentration, bilateral zygoneury occurring in both Lamellariacea and Cypraeacea and the pleural ganglia being closely adpressed to the cerebrals. In the reproductive system the sexes are separate in Lamellariacea and Cypraeacea and there is no bursa copulatrix in the female. In *Simnia* an open seminal groove runs from the male pore to the penis, but in *Trivia* and *Erato* and in the Lamellariacea the entire male duct is closed except for an accessory opening, perhaps of the nature of a safety valve, which puts the prostate into communication with the mantle cavity. The egg-cases are of relatively delicate construction since they are (in those species that have been observed) given protection by being sunk into the tissues of the ascidians upon which the adult animals normally feed; from them free-swimming echinospira larvae are produced (Lebour, 1935). *Simnia*, though clearly a member of the Cypraeacea in all other respects, is anomalous in its reproductive behaviour since the capsules which it produces are attached to its food on the surface (colonies of *Alcyonium* or *Eunicella*) and the larvae which emerge from them are ordinary veligers.

The picture of an idealized and generalized member of the Cypraeacea or Lamellariacea which emerges from this brief list of their outstanding characters is, clearly, of an animal distinctly different from that of the calyptraeacean. There is, without doubt, a much greater degree of relationship between *Capulus* and the other members of the Calyptraeacea than between it and the members of either the Lamellariacea or Cypraeacea. The echinospira larva is not a stage upon the presence or absence of which weighty arguments can rest: the Cypraeacea and Lamellariacea are akin without appeal to their common possession of that type of larva. *Simnia* is undoubtedly a cypraeacean even though it lacks an echinospira, and *Capulus* is equally a calyptraeacean even though it does possess one.

If this relationship be granted it is also true that the Cypraeacea, the Lamellariacea and the Calyptraeacea may all be regarded as lying close together in the great galaxy of the mesogastropods, and one obvious indication of this is the occurrence of the echinospira larva in all. Its presence, however, should not be taken to indicate more than that.

SUMMARY

The anatomy of *Trichotropis borealis* is described. The salient points are the possession of a spirally coiled shell with an operculum and a suboral proboscis, grooved dorsally, which curves to the right side of the head and collects food particles obtained from the water current in the mantle cavity. The alimentary

canal has small salivary glands and reduced jaws, but well developed glands in the mid-oesophagus and no crystalline style. The stomach is relatively simple with two large ducts opening from the digestive gland. Two main types of cell occur in this gland, one digestive in function, the other believed to be excretory. In the nervous system the cerebral and pleural ganglia are united, the supra- and suboesophageal ganglia have migrated well forwards and a zygoneury and a left dialyneury both occur. The animals are successive hermaphrodites and are protandrous. In the male phase a grooved penis occurs connected by a seminal groove with the genital pore in the mantle cavity. Rudiments of female organs are already present at this stage and later form a capsule gland with an albumen gland and receptaculum seminis. No bursa copulatrix occurs in the female and the penis disappears.

In *Capulus ungaricus* the animal has become limpet-like with a round shell and no operculum. Oesophageal glands have been lost but otherwise the gut and other soft parts conform to the same pattern as those of *Trichotropis*. Of *Crepidula fornicata* and *Calyptraea sinensis* the same may be said. Fusion in the nervous system is more extensive and feeding is carried out entirely by ciliary means; there is no proboscis.

Capulus possesses an echinospira larval stage. Comparison of its anatomy with that of members of the Lamellariacea and Cypraeacea, which (*Simnia* apart) also possess such a larva, shows that it is nearer to the other members of the Calyptraeacea. Possession of the echinospira, therefore, suggests a general relationship of these three superfamilies rather than a closer relationship of *Capulus* to the Lamellariacea and Cypraeacea than to the Calyptraeacea.

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J. Mar. biol. Ass. U.K. (1954), 33, 145–172
Printed in Great Britain

THE ECOLOGY OF BRITISH SPECIES OF *ENSIS*

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(Text-figs. 1–5)

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INTRODUCTION

In a recent paper describing the distinguishing characters of British species of *Ensis* (Holme, 1951), it was concluded that three valid species occur in British waters: *E. siliqua* (L.), *E. arcuatus* (Jeffreys) and *E. ensis* (L.). This paper is an attempt to analyse the ecological factors controlling the distribution of these species, particular attention being paid to the effect of soil grade.

In addition to those acknowledged in a previous paper, I would like to thank the following for their assistance: Dr A. G. Lowndes, Mr L. Birkett, Mr C. Edwards, and Mr O. D. Hunt. Specimens of *E. minor* were kindly supplied by Dr R. Tucker Abbott, U.S. National Museum.

I am indebted to the Director and Staff of the Station Biologique at Roscoff for facilities provided during a visit to the Station. Dr P. N. J. Chipperfield kindly placed at my disposal the research launch of I.C.I. Paints Division, Brixham, from which the dredgings in Torbay were made. I am grateful to

the Director of the National Institute of Oceanography for facilities provided on R.R.S. *Discovery II*, from which the bottom-sampling station in Aberporth Bay was worked.

THE HABITAT OF *ENSIS*

Ensis is found burrowing in sand at low-water mark of spring tides, and also occurs in shallow water offshore. On the beach, it burrows nearly vertically in the sand by means of a powerful foot, but it does not seem to possess a permanent burrow. The short siphons project just above the surface of the sand, when covered by water, and draw in water from just above the bottom. When disturbed, *Ensis* burrows rapidly into the sand, often emitting a jet of water into the air as it starts to burrow. Along a beach at low tide, *Ensis* may be located by the presence of keyhole-shaped depressions in the sand, which become more evident when the surface water has drained away, and also by the jets of water produced when they start to dig. It is possible to survey quite long stretches of beach in this way, only digging when a burrow is seen.

On a number of occasions the animals have been seen to come right out of the sand at low tide, and lie on the surface. This habit is also shared by certain other lamellibranchs living in the same habitat (e.g. *Spisula*, *Donax* and spiny *Cardium*).

On wave-exposed beaches, *Ensis siliqua* may be the only lamellibranch present, but in more sheltered areas the smaller *E. ensis* may be found, together with such species as *Venus striatula* (da Costa), *Donax vittatus* (da Costa), *Tellina fabula* Gmelin, *Gari fervensis* (Gmelin), *Mactra corallina cinerea* Montagu, and *Lutraria lutraria* (L.). The burrowing crab, *Corystes cassivelaunus* (Pennant), and the heart-urchin *Echinocardium cordatum* (Pennant) also occur in these localities.

Ensis arcuatus, which inhabits a coarser grade of soil, may be found with *Dosinia exoleta* (L.), *Tellina donacina* L., and the heart-urchins *Spatangus purpureus* O. F. Müller and *Echinocardium pennatifidum* Norman.

One or other of the species of *Ensis* occurs on practically every beach where there is sand of a sufficient depth and some slight protection from wave action. There may, however, often be none in small patches of sand between rocks: such sands are often highly reducing (see p. 158). *Ensis* does not occur in water of reduced salinity, although its absence from estuaries may sometimes be due to the lack of deposits of a suitable grade. A list of sandy beaches in which *Ensis* has not been recorded is given in Table VII (Appendix).

METHODS

In order to eliminate so far as possible the effect of characteristics peculiar to individual beaches, collections were made over as wide an area as possible. On the shore, collections have been made on most of the sandy beaches in S. Devon, S. Cornwall, and the Scilly Isles (Fig. 1). I have also received samples collected in various other parts of the British Isles. Additional samples have been obtained from certain beaches in Jersey and the north Finistère coast around Roscoff. A list of localities is given in Table VII (Appendix).

Grab or dredge collections are chiefly from Great West Bay (Holme, 1950), off Plymouth (some stations are in Holme, 1953), and in St Austell Bay off the Cornish coast (Fig. 5).

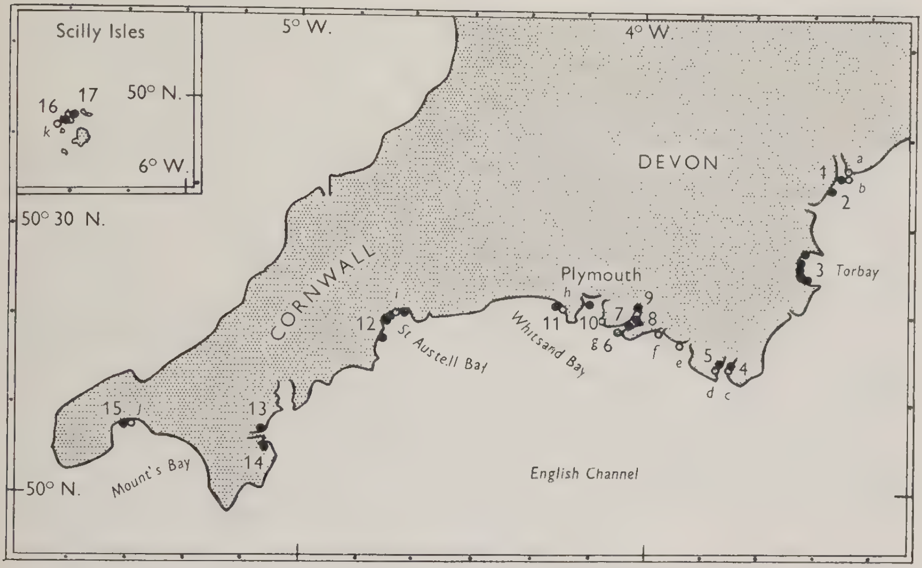

Fig. 1. Position of shore records (●) in Devon, Cornwall, and the Scilly Isles. Localities are numbered as follows: 1, Polesands; 2, Dawlish; 3, Torbay (see Fig. 5); 4, Salcombe, east; 5, Salcombe, west; 6, Cellars beach; 7, below Passage Wood; 8, Yealm sand bank; 9, Thorn Pt.; 10, Drake's Island; 11, Whitsand Bay; 12, St Austell Bay (see Fig. 5); 13, Helford; 14, Flushing Cove; 15, Marazion; 16, Tresco-Bryher channel; 17, Foreman's Island. Negative records (o): *a*, Maer Rocks, Exmouth; *b*, Polesands; *c*, south of Millbay, Salcombe; *d*, North Sands and South Sands, Salcombe; *e*, Borough Island; *f*, Mothecombe; *g*, Bovisand; *h*, Whitsand Bay; *i*, Charlestown (see Fig. 5); *j*, St Michael's Mount; *k*, Bryher.

At each locality a sample of the soil in which *Ensis* was living was taken for grade analysis. On the shore a sample from the top 5 cm. or so of soil was taken, all specimens taken within about a metre of the sample being considered to inhabit the same grade of soil. Samples were taken offshore either with the scoop-sampler (Holme, 1949*b*) or anchor-dredge (Forster, 1953). Where several hauls were made at a station, the soil sample was always taken from that in which *Ensis* was taken. There is little washing out of soil when the scoop-sampler is hauled up; but when using the dredge it was necessary to select a sample from an undisturbed portion of the dredge bag.

No accurate estimates of population density have been made. It is difficult to compare populations on different beaches, as tidal levels have to be taken into consideration. One is only sampling the fringe of the *Ensis* zone when collecting on the beach, and numbers are likely to change considerably over quite short vertical distances, so that the observed density may be much affected by the distance the tide recedes on a particular day. Offshore, the size of the sample, usually 0·5 m.^{2} or less, is too small for an assessment of density.

For comparative purposes, population density was classed as 'scarce', 'occasional', or 'common', as follows:

Scarce. Shore: a single specimen taken on a beach, or at most two or three, widely scattered. Offshore: a single specimen at an isolated station.

Occasional. Shore: several specimens on a beach, but only one need be taken in the vicinity of the soil sample. Offshore: two specimens at a station, or single specimens at two or more adjacent stations.

Common. Shore: density approximately one or more per m.^{2}. Offshore: more than two specimens at a station.

'Scarce' records may be only chance occurrences, and when computing the mean conditions in which a species is found may be disregarded. A conservative estimate was always made when there was any doubt as to the classification of any sample.

It is difficult to establish the absence of *Ensis* from a locality. On the shore one can observe that it is at most very scarce, but the offshore records cover too small an area to establish the absence of a species on a particular ground.

A grade analysis of soil samples was made as follows, the class units being based on the Wentworth system (2, 1, $\frac{1}{2}$, $\frac{1}{4}$ mm. etc.). The methods used and reliability of the results are described by Krumbein & Pettijohn (1938).

Samples were dried before storage, and for analysis were 'broken down' to a convenient size by dividing a flattened-out pile into quarters and taking opposite quarters. This operation was repeated until a sample of about 20 g. was obtained.

This was then treated with 20 vol. hydrogen peroxide on a hot plate for $\frac{1}{2}$ hr. to oxidize organic matter and break up aggregates, and then washed through a B.S.S. gauze sieve, with 0·124 mm. apertures.

The residue on the sieve was dried and screened through a set of B.S.S. sieves of the following apertures: 2·057, 1·003, 0·500, 0·251, 0·210, and 0·124 mm. (dimensions quoted by the manufacturers). The sieves were shaken mechanically by a machine which imparted a short but rapid to-and-fro motion, at the same time tilting them through a small angle to the horizontal by a slowly rotating eccentric. After 10 min. shaking a fairly accurate separation was obtained, and the fraction on each sieve was then weighed.

Material washed through the fine sieve after peroxide treatment was placed in a wide measuring cylinder having two marks, one 10 cm. above the other. Distilled water was added up to the top mark, and the material stirred into suspension. After allowing to settle for 1 min. 56 sec. (at 20° C.) the suspension was siphoned off down to the lower mark, the siphon having an upturned end so as not to disturb material which had settled. Distilled water was added up to the top mark, and the operation repeated until the siphoned-off material was almost clear. By this means a separation of particles over and under $\frac{1}{32}$ mm. was obtained. The residue in the cylinder was dried and added to that passing the finest of the sieves in the shaker. To the siphoned-off suspension was added a little alum solution and a few drops of acetic acid, to flocculate the particles. After a day or two this was filtered through a weighed filter-paper, washed, dried, and the weight of solid determined.

A separation was thus made at the following approximate particle diameters: 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{8}$ and $\frac{1}{32}$ mm. Large particles, over about 10 mm., were taken out of the sample before analysis, and were not reckoned with when calculating the percentage grade composition of the soil. Besides inorganic particles, the analysis includes shell and other calcareous material.

When more than one sample with the same species was taken from a beach, it was sometimes necessary to reduce the records to a single one to avoid bias to the mean from all collecting grounds in favour of a well-worked beach. When two or more samples did not differ by as much as 10% in any one grade, all but one sample was eliminated. The sample taken last on any one day was usually retained, as this was generally at the lowest level on the beach, and therefore more representative of the typical habitat, which often extends below low-water mark.

The results of mechanical analysis are conveniently expressed as a cumulative curve (Fig. 3, p. 156), with grade size on a logarithmic scale. The cumulative curve for any soil is independent of the class units, provided these are sufficiently closely spaced, and is therefore a more satisfactory method of representation than a histogram, in which the shape of the figure is dependent on the class units employed.

A convenient term for comparing sands which may differ particularly in the percentage of coarser particles is the arithmetic mean (see Krumbein & Pettijohn, 1938, p. 240). The mean of the upper and lower limits of each class is multiplied by the percentage weight in that class. The total of the products is divided by 100 to obtain the arithmetic mean. The method of calculation is shown below:

Grade size (mm.)	Mean grade (mm.)	Percentage in sample	Product
(4)–2	3·0	14·24	42·72
2–1	1·5	10·84	16·26
1–$\frac{1}{2}$	0·75	10·51	7·88
$\frac{1}{2}$–$\frac{1}{4}$	0·375	29·81	11·18
$\frac{1}{4}$–$\frac{1}{5}$	0·225	7·72	1·74
$\frac{1}{5}$–$\frac{1}{8}$	0·1625	16·90	2·75
$\frac{1}{8}$–$\frac{1}{32}$	0·0781	8·38	0·65
$\frac{1}{32}$–0	0·0156	1·60	0·02
		100·00	83·20

Mean = 83·20/100 = 0·832 mm.

The value of the arithmetic mean is clearly more affected by changes in the percentages in the coarser grades than in the finer ones.

GEOGRAPHICAL DISTRIBUTION

Many previous identifications have been incorrect, owing to failure to distinguish *E. arcuatus* as a separate species, and the following notes are therefore based almost entirely on specimens which I have seen. (The records in Ford, 1923, are known to be correct, as I have been able to confirm identifications from specimens in Mr Ford's shell collection.) All three specimens are generally distributed on the Atlantic coast of Europe, and *E. siliqua* and *E. ensis*, at least, occur in the Mediterranean. A list of localities is given below, and a more detailed list of places where living specimens have been taken is given in Table VII (Appendix).

E. SILIQUA

England: S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Studland Bay, Dorset[1]. **Scotland**: Aberdeen[1]; Fairlie, Ayrshire. **Isle of Man**: Port Erin Bay. **Wales**: Criccieth, Caernarvonshire[1]; Dale, Pembrokeshire; Tenby, Pembrokeshire[1]. **Ireland**: Benderg Bay, Co. Down. **France**: Grève de S. Michel, Finistère. **Italy**: Naples (from specimen department).

E. ARCUATUS

England: Scilly Isles; S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Pevensey Bay, Sussex[1]; Newton Haven, Northumberland. **Scotland**: Millport, Firth of Clyde (from specimen department). **Isle of Man**: Derbyhaven. **Wales**: Benllech, Anglesey[1]. **Ireland**: Portaferry and Benderg Bay, Co. Down. **Channel Isles**: Bordeaux Harbour, Guernsey; Jersey. **France**: Finistère.

E. ENSIS

England: S. Cornwall; S. Devon; Swanage Bay, Dorset[1]; Pevensey Bay, Sussex[1]; North Sea (52° 45′ N., 02° 44′ E.); off Cumberland coast (approx. 54° 23′ N., 03° 35′ W.). **Isle of Man**: Port Erin Bay. **Wales**: Aberporth, Cardiganshire. **Channel Isles**: Jersey. **France**: Finistère.

The Mediterranean form of *E. siliqua* is apparently smaller than the Atlantic form (Bucquoy, Dautzenberg & Dollfus, 1887–98, as var. *minor*), but specimens from Naples agree with the Atlantic form in the form of the fourth aperture papillae described by Holme (1951). The same authors give a photograph of *E. ensis* from Roussillon, on the French Mediterranean coast (pl. 73, fig. 5, as *E. ensis* var. *minor*; fig. 4 may be a small *E. arcuatus*).

There is insufficient data to fix the northern and southern limits of the three species, but they are not recorded from Iceland by Madsen (1949).

Four[2] species or forms of *Ensis* have been recorded from N. America: *E. directus* Conrad, *E. minor* Dall, *E. californicus* Dall (all listed in Dall, 1900), and the recently described *E. minor megistus* Pilsbry & McGinty (1943).

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[1] Empty shells.

[2] A fifth species has recently been described by Stillman Berry (1953).

E. directus resembles *E. arcuatus*, but the shell of the former is broader and more arcuate. In addition, Bloomer (1905) notes that the fourth aperture is bordered by several rows of papillae, whereas in *E. arcuatus* there is only a single row on each side. Pilsbry & McGinty have illustrations of both *E. minor* and *E. minor megistus*. The former closely resembles *E. ensis* in shell form, and may well be the same species. *E. minor megistus* is more slender and parallel-sided than either *E. minor* or *E. ensis*, and the anterior end is obliquely truncated, not rounded as in *E. ensis*. *E. minor megistus* does not correspond with any European species.

I have not been able to see specimens or figures of *E. californicus*.

Apart from the possibility that *E. minor* Dall is the same as *E. ensis*, I have no records of the British species from outside European waters (including the Mediterranean).

ENVIRONMENTAL FACTORS

The distribution of any organism is controlled by a complex of factors, each of which may act at a different point in the life history of the individual. Some factors may be too subtle to be detected by the methods employed, but an analysis of the more readily measurable factors should provide clues to the presence or nature of these other factors.

Any one factor interacts both with the organism and with its environment, and it is often difficult to distinguish whether the action of a factor is direct or indirect. Soil grade, for example, may influence distribution directly (see below), but it also has an indirect effect on the slope and stability of the beach, so affecting tolerance of wave action (see p. 158).

Soil Grade

Analyses of soil grade for the three species are given in Tables I–III. In these tables the samples have been grouped according to population density as 'scarce , 'occasional' or 'common', as defined on p. 148. Fig. 2 shows the percentage in each grade in histogram form, and the distribution of arithmetic means is given in Fig. 4. The mean of 'occasional' and 'common' samples only is shown as a cumulative curve in Fig. 3.

E. siliqua occurs in sands of fairly fine grade, in which particles between 0·21 and 0·0313 mm. preponderate. This species is not confined to sands of any particular grade composition within these limits, but seems to avoid those in which particles over 0·5 mm. form more than about 5 % of the total. The percentage of silt ($<0{\cdot}0313$ mm.) is always small, and is usually less than 1 %. *E. siliqua* is thus characteristic of ordinary 'clean' beach sands.

E. ensis occurs in soils of a grade similar to that in which *E. siliqua* is found. The histograms showing percentage weights in the different grades are very similar (Fig. 2), as is the distribution of arithmetic means (Fig. 4). Only eight of the *E. ensis* samples also contain *E. siliqua*, so the coincidence of the

TABLE I. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS SILIQUA* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.)							
			>2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Polesands	*47*	0·236	0·4	1·0	1·3	15·2	14·9	64·8	2·3	0·05
Saltern Cove	*53*	0·245	0	0·2	2·1	33·1	11·9	40·6	11·9	0·22
Salcombe, E.	*63*	0·646	3·1	17·4	25·5	10·2	5·3	22·7	15·0	0·76
Drake's Is. (C)	*172*	0·620	7·9	15·9	7·2	5·1	3·3	21·1	37·5	1·95
Drake's Is. (B, C)	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Drake's Is.	*176*	0·103	0·1	0·1	0·3	1·7	1·3	15·4	79·7	1·36
Whitsand Bay	*117*	0·276	0	0·1	1·0	44·2	22·2	31·0	1·5	0·07
Porthpean	*61*	0·701	11·1	10·6	5·8	21·5	12·1	34·7	4·0	0·31
Helford River (C)	*41*	0·268	3·3	1·1	0·9	5·3	5·9	58·9	22·5	2·20
Flushing Cove	*58*	0·110	0	0·1	0·5	6·0	1·8	10·2	78·1	3·35
Mean		0·348	2·6	4·8	4·9	18·0	9·0	33·0	26·7	1·22
OCCASIONAL										
Dawlish	*74*	0·304	5·0	0·6	0·3	1·2	1·3	75·4	15·9	0·33
Salcombe, E. (B)	*6*	0·254	0·1	0·8	2·7	28·8	16·2	40·8	10·4	0·25
Par	*66*	0·287	0·2	0·1	3·2	44·9	14·3	31·8	5·2	0·38
Pentewan	*136*	0·222	0	0·3	1·3	18·9	17·9	57·1	4·4	0·16
Helford River	*38*	0·183	1·1	0·2	0·2	2·1	3·5	70·8	20·5	1·63
Helford River (B, C)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Dale Flats	*25*	0·100	0·2	0·1	0·1	0·3	0·2	18·5	79·3	1·38
Grève de S. Michel	*155*	0·085	0	0	0·1	0·1	0·3	6·1	93·0	0·35
Torbay[1]	*169*	0·140	0·3	0·7	0·6	0·6	0·3	45·1	51·8	0·61
Mean		0·198	0·9	0·4	1·0	11·4	7·0	45·8	32·8	0·71
COMMON										
Torr Abbey Sands	*139*	0·102	0·2	0·1	0	0·2	0·3	19·1	79·4	0·64
”	*141*	0·200	2·7	0·4	0·3	0·5	0·5	38·4	56·3	0·80
Paignton	*10*	0·187	0	0·6	1·6	5·9	3·5	79·5	8·7	0·20
Goodrington	*59*	0·287	2·0	3·7	3·5	5·0	3·0	67·3	15·2	0·34
”	*60*	0·169	0	0	0·1	3·4	7·4	82·6	6·4	0·17
Broadsands (C)	*2*	0·110	0	0	0·3	0·3	0·3	34·2	64·4	0·47
Broadsands (C)	*11*	0·199	1·3	0·7	0·5	1·0	0·8	77·8	17·7	0·29
Elbury Cove (C)	*106*	0·117	0·3	0	0	0·8	0·7	33·9	63·5	0·83
Par	*68*	0·096	0	0	0·1	0·4	0·5	20·0	77·6	1·40
Duporth	*62*	0·211	0·5	1·5	1·2	7·2	6·3	68·2	14·5	0·60
Flushing Cove (C)	*55*	0·169	0	0·1	0·4	11·0	9·3	49·5	28·5	1·28
Marazion	*52*	0·110	0	0·1	0·1	0·1	0·1	34·8	64·5	0·28
Dale Flats	*73*	0·139	0·1	0·1	0·1	0·3	0·1	66·1	32·1	1·11
Fairlie Sands	*18*	0·278	0	0	0·6	49·2	15·1	33·3	1·8	0·20
Torbay[1] (C)	*167*	0·089	0	0·1	0·1	0·2	0·3	10·8	87·7	0·77
Plymouth[1]	*22*	0·193	1·8	0·3	0·3	2·7	2·1	55·1	36·2	1·61
Mean		0·166	0·6	0·5	0·6	5·5	3·1	48·2	40·9	0·69

[1] Offshore records. (B), with *E. arcuatus*; (C), with *E. ensis*.

Table II. Grade-Composition of Soils in which *Ensis arcuatus* occurs

	Sample no.	Arith. mean (mm.)	Grade (mm.)							
			> 2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	< 0·0313 (s)
Scarce										
Salcombe, W.	*64*	0·739	14·4	9·6	3·1	8·8	11·4	47·7	4·5	0·58
,,	*65*	1·453	33·2	22·7	5·9	6·7	4·0	19·4	7·9	0·28
Yealm River	*69*	2·144	60·8	16·0	8·0	2·7	0·4	2·6	5·7	3·77
,,	*171*	0·772	15·2	3·5	5·8	47·0	9·8	11·4	5·8	1·67
Helford River	*164*	0·196	1·3	0·7	0·7	3·9	5·1	55·7	29·6	2·97
Helford River (A, C)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Flushing Cove	*56*	0·832	14·2	10·8	10·5	29·8	7·7	16·9	8·4	1·60
,,	*57*	0·188	0·2	0·2	0·8	15·0	11·2	42·2	28·7	1·71
St Aubin's Bay	*15*	0·407	5·1	5·7	4·3	13·7	6·0	25·0	40·0	0·31
Térénès (C)	*149*	0·245	0·8	1·1	1·1	15·5	17·3	60·0	3·8	0·46
Plymouth[1] (C)	*31*	0·177	0·1	0·4	1·1	6·1	8·6	63·7	19·8	0·22
Mean		0·669	13·3	6·5	3·8	14·1	8·2	37·4	15·4	1·36
Occasional										
Salcombe, E. (A)	*6*	0·254	0·1	0·8	2·7	28·8	16·2	40·8	10·4	0·25
Yealm River	*70*	0·597	10·6	3·3	2·9	29·9	18·4	32·8	2·0	0·26
,,	*71*	0·667	7·6	9·8	15·8	32·1	7·6	18·6	7·9	0·73
Drake's Is. (A, C)	*173*	0·433	0·7	2·2	8·0	81·8	4·4	1·8	0·7	0·45
Drake's Is.	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Foreman's Is.	*128*	0·866	16·2	9·3	8·3	36·9	6·5	11·1	10·7	1·21
Grouville Bay (C)	*16*	0·215	0	0·4	3·3	13·0	12·7	61·8	8·7	0·18
Goulven	*157*	0·208	0·2	0·4	2·2	16·0	7·0	56·7	17·6	0·05
Perharidy	*159*	0·726	7·3	7·8	19·5	64·0	0·6	0·6	0·1	0·05
Mean		0·471	4·7	3·9	7·4	37·8	9·4	28·3	8·0	0·57
Common										
Salcombe, E.	*3*	0·545	0·3	5·0	45·6	21·4	4·3	14·8	8·3	0·35
Salcombe, E. (C)	*12*	0·482	1·0	13·2	19·4	8·7	4·0	31·0	21·4	1·25
Yealm River	*1*	1·307	25·2	19·0	19·7	29·3	1·7	2·2	1·7	1·19
Tresco	*50*	1·242	10·6	42·5	32·0	10·6	2·1	1·9	0·3	0
Tresco-Bryher channel	*120*	0·961	11·7	21·0	21·1	27·7	6·3	10·7	1·4	0·13
,,	*121*	1·267	25·7	16·0	15·9	30·9	5·0	5·5	0·8	0·19
,,	*122*	0·978	10·6	23·3	20·6	38·0	3·2	3·8	0·4	0·19
Foreman's Is.	*126*	1·020	20·5	9·1	11·0	41·7	7·6	8·1	1·7	0·40
,,	*127*	0·641	5·5	8·5	13·9	58·7	6·8	5·9	0·7	0·17
,,	*129*	0·964	13·9	15·5	21·2	36·3	4·5	4·8	2·4	1·50
Tresco-Bryher channel	*130*	1·138	19·1	16·8	23·3	34·0	3·5	2·4	0·6	0·27
,,	*131*	0·622	3·3	9·9	21·7	47·7	9·2	7·7	0·3	0·13
Newton Haven	*19*	0·195	1·1	0·6	1·2	3·3	1·5	64·7	27·3	0·25
Grouville Bay	*9*	0·314	0	0·3	6·3	48·4	17·1	25·2	2·7	0·12
Goulven	*158*	1·612	30·2	33·0	21·0	14·1	0·6	0·7	0·4	0·04
Mean		0·886	11·9	15·6	19·6	30·0	5·2	12·6	4·7	0·41

[1] Offshore records.

(A), with *E. siliqua*; (C), with *E. ensis*.

TABLE III. GRADE-COMPOSITION OF SOILS IN WHICH *ENSIS ENSIS* OCCURS

	Sample no.	Arith. mean (mm.)	Grade (mm.)							
			>2·0 (I)	2·0–1·0 (II)	1·0–0·5 (III)	0·5–0·25 (IV)	0·25–0·21 (V)	0·21–0·125 (VI)	0·125–0·0313 (fs)	<0·0313 (s)
SCARCE										
Torr Abbey Sands	*140*	0·143	1·1	0·1	0·1	0·3	0·4	35·9	61·4	0·82
Elbury Cove	*105*	0·125	0	0	0	2·5	1·2	45·6	49·6	1·02
Elbury Cove (A)	*106*	0·117	0·3	0	0	0·8	0·7	33·9	63·5	0·83
Salcombe, W.	*163*	0·836	21·1	6·6	0·8	2·9	3·9	32·6	30·9	1·17
Yealm River	*72*	1·164	28·1	10·2	4·6	20·0	8·3	20·6	7·4	0·76
Drake's Is. (A)	*172*	0·620	7·9	15·9	7·2	5·1	3·3	21·1	37·5	1·95
Drake's Is.	*174*	0·225	2·3	1·6	1·1	4·9	4·3	34·9	49·1	1·87
Flushing Cove	*54*	0·142	0	0·3	1·2	7·9	3·3	29·2	55·7	2·45
Grouville Bay	*7*	0·168	0·3	0·1	0·1	2·0	3·3	82·7	11·4	0·12
Grouville Bay (B)	*16*	0·215	0	0·4	3·3	13·0	12·7	61·8	8·7	0·18
Térénès	*147*	0·267	1·3	3·7	3·1	6·1	3·5	64·8	16·7	0·82
Térénès (B)	*149*	0·245	0·8	1·1	1·1	15·5	17·3	60·0	3·8	0·46
Goulven	*156*	0·194	0	0·1	2·0	16·9	7·1	47·9	25·9	0·14
Plymouth[1]	*116*	0·185	0·1	0·1	0·6	9·2	17·4	57·1	13·8	1·80
Plymouth[1]	*113*	0·272	2·4	3·2	3·2	3·4	2·8	51·6	30·1	3·30
Plymouth[1]	*32*	0·158	0	0·2	0·7	2·9	4·8	69·6	19·8	2·12
Mean		0·317	4·1	2·7	1·8	7·1	5·9	46·8	30·3	1·24
OCCASIONAL										
Broadsands (A)	*2*	0·110	0	0	0·3	0·3	0·3	34·2	64·4	0·47
Broadsands (A)	*11*	0·199	1·3	0·7	0·5	1·0	0·8	77·8	17·7	0·29
Salcombe, E. (B)	*12*	0·482	1·0	13·2	19·4	8·7	4·0	31·0	21·4	1·25
Drake's Is. (A, B)	*175*	0·272	0	1·0	4·1	37·7	10·9	30·1	14·3	1·93
Helford River	*24*	0·094	0	0·3	0·1	0·4	0·6	22·0	60·6	16·00
,,	*36*	0·177	0·7	0·1	0·2	2·5	3·9	73·1	18·6	0·83
,,	*40*	0·226	2·7	1·2	1·2	2·7	1·8	45·1	40·3	5·17
Helford River (A)	*41*	0·268	3·3	1·1	0·9	5·3	5·9	58·9	22·5	2·20
Helford River (A, B)	*165*	0·208	0·8	1·2	0·8	6·0	8·6	66·4	15·0	1·34
Flushing Cove (A)	*55*	0·169	0	0·1	0·4	11·0	9·3	49·5	28·5	1·28
Flushing Cove	*162*	0·147	0	0·4	0·6	6·0	4·7	41·9	45·3	1·26
Penpoull	*145*	0·443	7·4	4·5	2·8	3·4	1·8	62·9	16·1	1·14
Great West Bay, St. 12[1]	*27*	0·161	0·2	0·2	0·7	3·4	1·4	69·0	24·8	0·40
Great West Bay, St. 17[1]	*28*	0·136	0·1	0·3	0·9	5·6	2·2	33·8	53·6	3·45
Great West Bay, St. 16[1]	*29*	0·157	0·1	0·2	0·9	7·4	3·9	48·2	38·8	0·54
Torbay, St. 9[1]	*167*	0·089	0	0·1	0·1	0·2	0·3	10·8	87·7	0·77
Torbay, St. X[1]	*170*	0·080	0	0	0·1	0·2	0·1	1·4	97·3	0·91
Plymouth[1] (B)	*31*	0·177	0·1	0·4	1·1	6·1	8·6	63·7	19·8	0·22
St Austell Bay, St. 3[1]	*33*	0·145	0	0·2	1·0	5·5	5·3	46·4	30·9	10·74
St Austell Bay, St. 4[1]	*34*	0·098	0	0·1	0·6	2·0	1·6	19·6	60·9	15·29
St Austell Bay, St. 6[1]	*35*	0·109	0	0·4	1·3	4·2	1·2	7·0	80·2	5·71
Irish Sea[1]	*14*	0·203	0	0·7	1·6	12·2	8·8	65·0	10·6	1·22
Mean		0·189	0·8	1·2	1·8	6·0	3·9	43·5	39·5	3·29
COMMON										
St Aubin's Bay	*21*	0·180	0·5	1·8	1·8	8·2	5·1	20·3	61·9	0·42
Great West Bay, St. 19[1]	*30*	0·313	1·8	1·7	5·5	26·7	8·3	37·4	14·0	4·63
Aberporth Bay[1]	*160*	0·300	0·5	0·3	0·6	53·4	21·6	12·8	7·4	3·33
Mean		0·264	0·9	1·3	2·6	29·4	11·7	23·5	27·8	2·79

[1] Offshore records. (A), with *E. siliqua*; (B), with *E. arcuatus*.

histograms is not due to both being made up of the same set of samples. *E. ensis* is able to tolerate a slightly higher percentage of coarse material over 0·5 mm., but typically occurs where there is 3% or less. *E. ensis* is found in soils with a silt percentage ranging from 0·1 to 16%, the mean being about 3%. One might expect the mean silt percentage for this species to be rather higher than for *E. siliqua*, since most of the samples are from sheltered

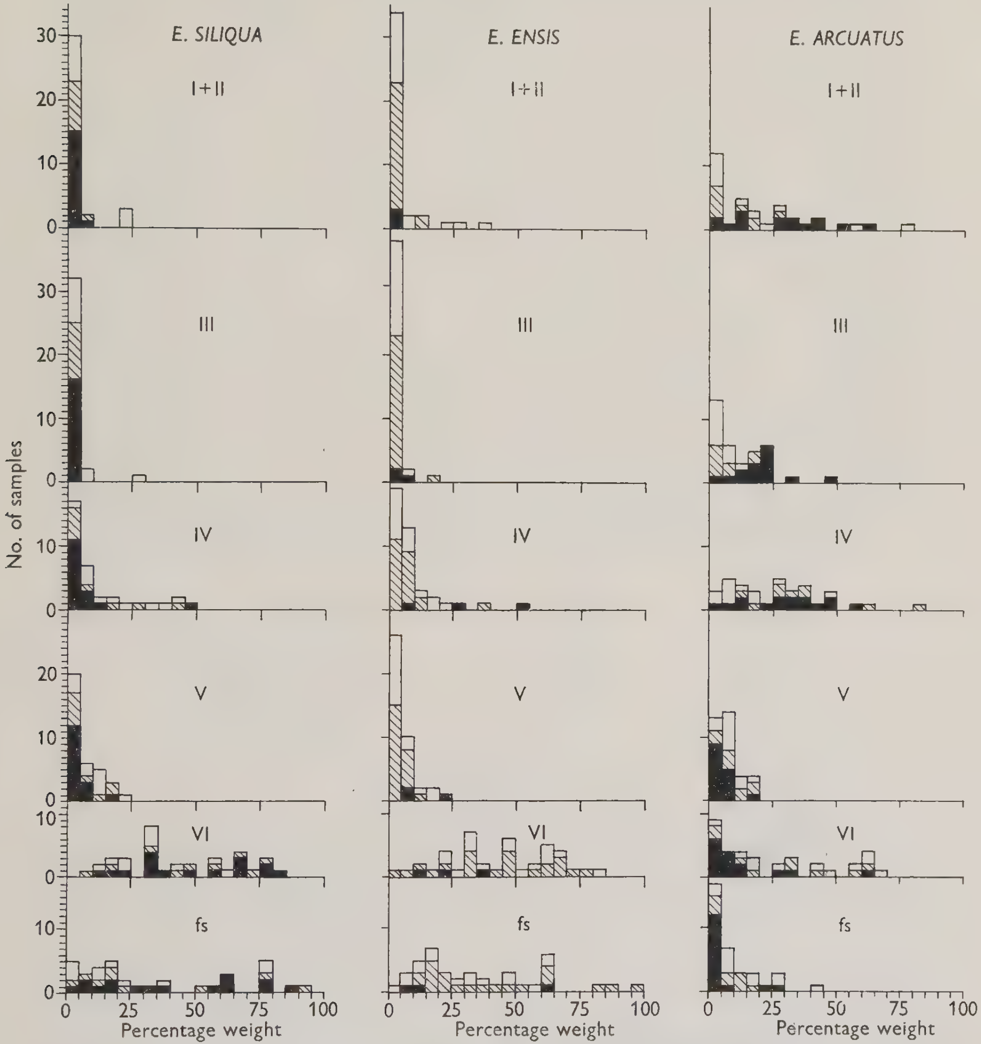

Fig. 2. Diagram summarizing the soil analyses given in Tables I–III. The percentage weights in each grade have been grouped in 5% units, and the number of samples in each 5% group are shown in histogram form. The silt grade has been omitted. Samples are represented thus: ■, common; ▨, occasional; □, scarce.

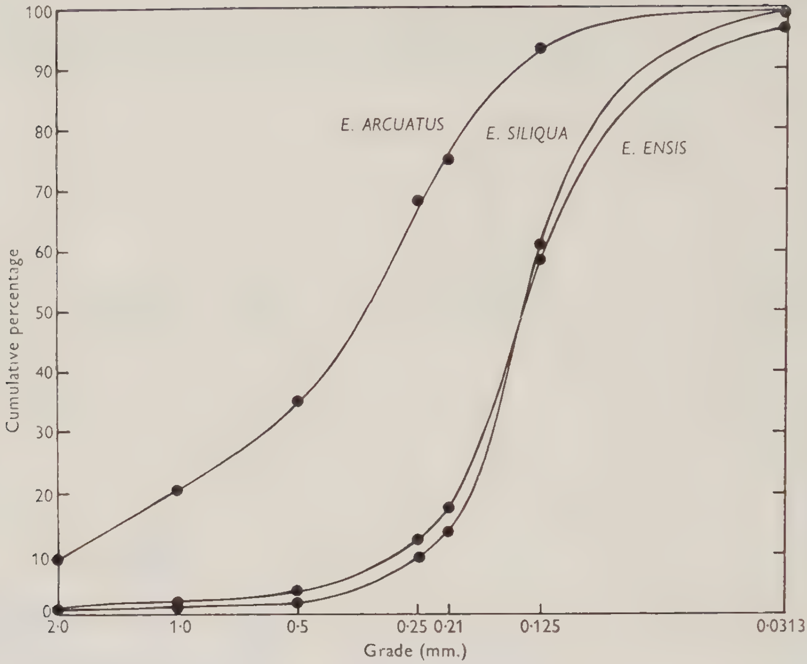

Fig. 3. Cumulative curves of the mean soil grade of 'common' and 'occasional' samples.

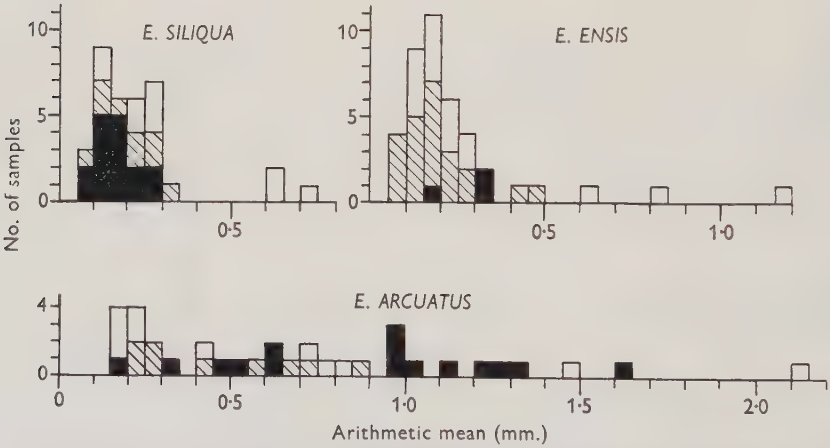

Fig. 4. The arithmetic mean of soils inhabited by the three species. The mean is grouped in units of 0·05 mm. Samples are represented thus: ■, common; ▨, occasional; □, scarce.

localities or from offshore, whereas the silt content is usually higher than on an open beach, but the occurrence of *E. ensis* in three samples containing over 10% silt indicates that it does have a greater silt tolerance.

E. arcuatus inhabits soils of a coarser grade than the other two species. The arithmetic mean of the soils ranges from less than 0·2 mm. to over 1·5 mm. In fine-grade soils its distribution overlaps that of *E. siliqua* and *E. ensis*, and it occurs with either or both of these species on certain beaches. Sands inhabited by *E. arcuatus* typically contain about 35% over 0·5 mm., and naturally show a correspondingly lower percentage in the finer grades. It is therefore not possible to say whether the distribution of this species is correlated with the presence of coarse or the relative scarcity of fine particles.

The density of *Ensis* populations is usually greater in sands approaching the 'mean' grade of the samples for that species. This is clear from an inspection of Figs. 2 and 4. In particular, population densities of *E. siliqua* and *E. ensis* are less in soils coarser than the mean. The densities on different beaches cannot readily be related to the grade of deposit, as so many other factors may be concerned. In St Austell Bay, however, there are a number of open beaches within a few miles of each other (Fig. 5), on which the populations of *E. siliqua* are very different. These differences may be correlated with the varying grade of beach deposit, but the effect could be an indirect one due to the greater disturbance by waves on beaches of coarse sand (see p. 158).

Beach	Arith. mean (mm.)	Population density
Porthpean	0·701	Scarce
Par (nr. Callyvador rocks)	0·287	Occasional
Pentewan	0·222	Occasional
Duporth	0·211	Common
Par (E. end beach)	0·096	Common

Nature of the Soil Particles

The sands in which *Ensis* occurs are usually predominantly of quartz grains, although the percentage of calcareous material is often high.

Sandy beaches in Devon and Cornwall are usually discontinuous, being separated by rocky headlands, so that little movement of material along the coast can occur. The sand grains on any beach are probably formed largely from rocks in the vicinity, together with material washed in from offshore. The size, shape, and surface texture of the quartz grains will depend on the rocks from which they are derived (Hatch & Rastall, 1952, pp. 86–90), although some subsequent degree of rounding may be achieved on exposed beaches.

Between Paignton and Exmouth the cliffs are mainly of New Red Sandstone, which produces fine-grade quartz grains with some degree of rounding. Much of the coastline between Torbay and Mount's Bay is formed of Devonian slates and grits: these produce more angular grains, similar to the Polzeath sand figured by Wilson (1948, plate XVII). Grains derived from the chain of granite bosses extending through Devon and Cornwall are probably found in most of these sands. Where there are china clay workings, kaolin and mica may be carried down to the sea in large quantities, contributing both to littoral and offshore deposits. This is particularly noticeable in St Austell Bay. The granite of the Scilly Isles produces a coarse-grained quartz sand, in which *E. arcuatus* is abundant.

In Jersey and Brittany the local granite and metamorphic rocks produce quartz grains of a much smaller size than in Scilly. Pruvot (1897) has described the geology and deposits of the N. Brittany coast, and has shown that many of the beaches have a high calcium carbonate content.

There is no evidence that any species of *Ensis* is restricted to sand of a particular mineralogical character, degree of roundness, or surface texture. Wilson (1953) suggests that an organic coating on certain sands affects the settlement of *Ophelia* larvae. Most sands probably possess an organic coating of some sort, but this matter has not been investigated. Certain sands from the Scilly Isles have a conspicuous green coating on the grains, which may be of algal or fungal nature.

Reducing Conditions in the Sand

Ensis typically occurs in sands which are not black below the surface, indicative of reducing conditions. Where seaweed or other organic matter gets buried in and incorporated in the sand, resulting in a black layer containing ferrous sulphide, *Ensis* is absent. The absence of *Ensis* from beaches at Mothecombe and Bovisand, near Plymouth, is probably due to such conditions. *Ensis* can tolerate sands which are slightly reducing, in which there is a grey layer below the surface, however. Such conditions occur on beaches of firm fine sand in which the organic content is not high, but there is little circulation of water. *E. siliqua* has been found in 'grey' sands at Torr Abbey, Elbury, S. Michel, etc.

Wave Exposure

On exposed beaches where the sand is continually churned by waves, *Ensis* is absent. Such beaches are typically barren. *E. siliqua* is only found where there is at least slight shelter from wave action. Tolerance of wave action seems to be dependent on the stability of beach deposits. King (1951) has shown that on an exposed beach the depth of disturbance during storms is unlikely to exceed 20 cm., but if wholesale transport of material occurs the sand may be disturbed to a much greater depth. The depth of disturbance in a coarse sand is greater than in a fine one, on account of the steeper slope of beaches of coarse material.

Although *E. siliqua* is capable of rapid burrowing, it is sometimes washed inshore in large numbers after storms, as are the other species. In very exposed areas it is only able to live below low-water mark, where wave disturbance is less severe (e.g. in Bigbury Bay). Some of those washed on to an exposed beach after a storm may burrow into the sand and establish a temporary foothold until disturbed by the next storm. This may account for occasional specimens found on the beach at Whitsand, also on the Polesands, where wave action is not extreme, but the bank is too shifting for *Ensis* to persist. On the other hand, *E. siliqua* is found in large numbers on the exposed beach at Marazion, in Mount's Bay. Its occurrence may be due to the sand there being fine and gently sloping, so that the depth of disturbance is minimized. The arithmetic mean of a sample from Marazion was 0·110 mm., as against 0·276 mm. in Whitsand Bay.

E. arcuatus seldom occurs on open beaches in south-west England, and this may be due to the relatively greater depth of disturbance for a given wave height on beaches of the coarse sand which it inhabits. It is confined to sheltered harbours and estuaries where accumulations of coarse material occur. It is also found in relatively sheltered beaches between the islands in Scilly.

Although occurring in sands of a similar grade to those occupied by *E. siliqua*, *E. ensis* is absent from the more exposed beaches on which the former occurs. In such exposed areas, it may occur only below low-tide mark (e.g. St Austell Bay, Fig. 5). In Jersey and Finistère, however, it occurs on exposed beaches (see pp. 162–3).

It is difficult to obtain an objective estimate of the severity of wave action on a beach. On nearly all beaches on which *Ensis* occurs, wave action may at times be severe, and a comparison of beaches is perhaps best made in relation to the normal conditions which prevail, rather than to the extreme conditions when a storm is blowing from an unusual quarter. On the basis of the normal wave exposure, a tentative classification has been made as follows:

(1) Beaches fully exposed to prevailing wind and swell (from south-west).
(2) Beaches with some slight shelter from prevailing wind and swell.
(3) Beaches sheltered from prevailing wind and swell, but exposed to waves from another direction.
(4) Beaches in bays which are sheltered from prevailing wind and swell.
(5) Sheltered beaches in harbours and estuaries.

Such a classification cannot take into account individual differences due to submarine topography, etc., but in the absence of detailed observations is the nearest approximation which can be achieved.

The occurrence of species of *Ensis* in relation to wave-exposure is summarized in Table IV. *E. siliqua* is found in all five categories, but *E. ensis* and *E. arcuatus* are more or less confined to groups 4 and 5. Notable exceptions are the occurrence of these last two species on exposed beaches on the south side of the Channel.

The occurrence of *E. siliqua* and *E. ensis* in St Austell Bay and Torbay is shown in Fig. 5. St Austell Bay is sheltered from the west, but is open to the south and south-east. *E. siliqua*, only, occurs on the beaches there, exposure being too great for *E. ensis*, which occurs offshore. (*E. siliqua* has not been recorded offshore, where it no doubt occurs in shallow water.)

TABLE IV. OCCURRENCE OF *ENSIS* IN RELATION TO WAVE EXPOSURE

(Names in italics are of beaches on the south side of the Channel. +, 'common' or 'occasional' records; (+), 'scarce' records. Samples from certain areas (e.g. Scilly Isles) are lumped together. ⋆, probably washed in from offshore.)

Exposure	Beach	*siliqua*	*arcuatus*	*ensis*
1	Whitsand	(+)⋆	–	–
	Marazion	+	–	–
2	*St Aubin's Bay*	–	(+)	+
	Goulven	–	+	(+)
3	Polesands	(+)⋆	–	–
	Dawlish	+	–	–
	Paignton	+	–	–
	Goodrington	+	–	–
	Saltern	(+)	–	–
	Par	+	–	–
	Duporth	+	–	–
	Porthpean	(+)	–	–
	Pentewan	+	–	–
	S. Michel	+	–	
	Perharidy	–	+	–
	Grouville	–	+	(+)
4	Torr Abbey	+	–	(+)
	Broadsands	+	–	+
	Elbury	+	–	(+)
	Cellars Beach	–	+	(+)
	Scilly Is.	–	+	–
	Dale	+	–	–
	Térénès	–	(+)	(+)
5	Salcombe	+	+	+
	Yealm (excl. Cellars Beach)	–	+	–
	Drake's Is.	(+)	+	+
	Helford	+	(+)	+
	Flushing	+	(+)	+
	Penpoull	–	–	+

Torbay is more sheltered, being open only to the east. *E. siliqua* occurs on all sandy beaches, and in shallow water offshore. *E. ensis* is absent from beaches in the middle of the bay, being found only at Torr Abbey Sands, in the north-west corner, and Broadsands and Elbury in the south-west corner. These beaches are probably a little more sheltered than those in the middle of the bay.

Currents

On open beaches the effects of currents are negligible in comparison with the water movements produced by breaking waves. No correlation has been found between the occurrence of *Ensis* and the strength of currents, although these have an indirect effect by their action on the distribution of particles, particularly in sheltered waters.

Depth Distribution

On the shore all species occur from about MLWST downwards, often increasing in number toward extreme low-water mark.

E. siliqua extends seawards to a depth of *c.* 20 m. on suitable substrata. It seems to be most common at extreme low water and just below. Several specimens were taken in Bigbury Bay in 20 m., and it has also been taken in the anchor-dredge in Torbay in depths of 1·6–6·9 m., where it is common. Other specimens have been taken in shallow water in Whitsand Bay and Plymouth Sound.

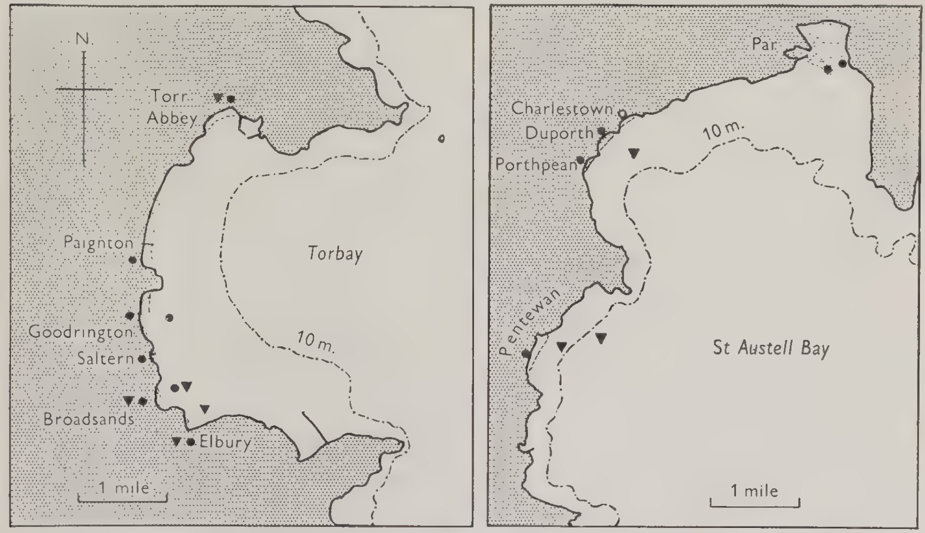

Fig. 5. Charts of Torbay and St Austell Bay, showing the distribution of *E. siliqua* (●) and *E. ensis* (▼). Beach samples are indicated on the land masses, offshore samples on the sea, the latter in their approximate positions. A negative record for Charlestown is also shown. Sandy beaches are stippled. The 10 m. depth contour is shown.

E. ensis occurs in small numbers at LWST in south-west England, but is much more common in depths of *c.* 10 m. (see Table VII, Appendix, and Ford, 1923). Single specimens have been taken in depths of 58·5 m. and 60 m. off Plymouth (Holme, 1953).

E. arcuatus occurs at LWST and has been recorded in 14·5 m. in Whitsand Bay. It is sometimes taken when dredging in the Eddystone shell gravel in *c.* 42 m. Ford (1923) records it from a number of stations in Plymouth Sound.

Moore (1936) has shown that *Echinocardium cordatum*, which lives in a similar habitat to *Ensis siliqua*, settles below low-water mark, and may subsequently migrate into the intertidal region. There is no evidence of a similar migration in *Ensis*. Although small specimens of *Ensis* were not often dug on the shore, they might easily have been missed since sieving methods were not employed. On those days when *Ensis* came out of the sand at low tide, small specimens of a size seldom taken when digging were sometimes seen. These would normally be missed because their burrows are inconspicuous, and they would rarely be taken during random digging. The only movements of populations so far recorded occur when *Ensis* are washed in during storms (p. 158).

Biotic Factors

The inhabitants of a sandy beach are usually rather sparse, and it is difficult to see that filter-feeding organisms like *Ensis* are in competition with one another for a food supply that is being continually renewed by water movements.

Ensis is found in habitats in which there are great differences in the quantity of food available. The amount of organic detritus in suspension over an open beach is usually small, while in enclosed bays and estuaries large quantities may be in suspension, particularly where there is much *Zostera*. The quantity of detritus probably also varies with depth of water and distance offshore. Unless *Ensis* is a highly selective feeder, there seems no reason to suppose that its distribution is correlated with its food supply.

It is probable that some toll of larvae or newly settled spat is inflicted by filter or deposit feeders already established on a beach, but this is more likely to affect the density rather than absolute distribution. Similarly, fish probably take a heavy toll, both of small whole individuals and of the siphons and posterior end of adults. I cannot recall ever having seen an empty shell of *Ensis* which had been drilled by the predatory gastropod *Natica*.

A heavy mortality must result when *Ensis* comes out of the sand at low tide (see p. 146). While some probably survive the exposure and burrow in again when the tide returns, many must be eaten by gulls.

Distribution on the Southern Side of the English Channel

The distribution and abundance of *Ensis* on beaches in Finistère and Jersey is rather different from that in south-west England. Although the grade of soil occupied by each of the three species is much the same as in England (Tables I–III), there are differences in tolerance of wave-exposure (Table IV).

In Jersey the tidal range is large, being 32·5 ft. (9·91 m.) at spring tides. The beaches are gently sloping in most places, so that the distance between high and low water may be as much as a kilometre or more. St Aubin's Bay and Grouville Bay are relatively exposed, but on such gently sloping beaches, mostly of firm fine sand, the depth of wave disturbance cannot be great (cf. King, 1951). In the middle of St Aubin's Bay (near Beach Rock), wave action is so severe as to exclude burrowing lamellibranchs, although small clumps of eel-grass (*Zostera*), indicating fairly stable conditions were found. Close to St Aubin's Fort, on the west side of the bay, where there is more shelter, *E. ensis* was found in some abundance, together with several *E. arcuatus*. This is the only beach on which *E. ensis* has been taken in any numbers. *Solen marginatus* Montagu also occurs, but at a slightly higher level on the beach.

In Grouville Bay, which is open to the east and only sheltered in this direction by isolated reefs, *E. arcuatus* and *E. ensis* were quite common near S. Etac, as was *Solen marginatus*.

These beaches differ from those in south-west England in the presence of *Ensis ensis* and *E. arcuatus* under more exposed conditions, in the absence or rarity of *E. siliqua* (none was found during 3 days' collecting), and in the abundance of *Solen marginatus*, which is restricted to sheltered conditions in muddy sand of estuaries, etc., in England.

E. arcuatus and *E. ensis* are fairly common on beaches in N. Finistère, but *E. siliqua* is not, being only found on one of the beaches investigated, S. Michel. Here *E. siliqua* was present in small numbers on the fairly exposed beach, together with many specimens of the pod-razor, *Pharus legumen major* Bucquoy, Dautzenberg & Dollfus, and *Mactra corallina cinerea*, in great abundance. *Ensis ensis* was not taken on this beach. Both *E. arcuatus* and *E. ensis* were found on the relatively sheltered beach at Térénès. At Goulven, a wide sandy beach some 5 km. across, the sand at the eastern, exposed, end is almost barren (one *Corystes cassivelaunus* was the only burrowing animal found). Towards the middle of the bay there are patches of *Zostera*, under conditions similar to those in which it was found in St Aubin's Bay, but no burrowing lamellibranchs were taken. In the middle of the bay there is slightly more shelter from the headland on the west side of the bay, but the sand is thrown up into ridges at right angles to the direction of wave incidence, evidence of considerable wave disturbance. On these ridges were found *Callista chione* (L.), *Donax variegatus* (Gmelin), *Spisula* sp., and *Ensis ensis* and *E. arcuatus* in small numbers. *Solen marginatus* is rare or absent on exposed beaches in Finistère, but is found in sheltered areas, as at Penpoull, in a habitat similar to that in which it occurs in S.W. England.

E. siliqua is also scarce farther south: in a survey of sandy beaches near Quiberon (S. Brittany), Prenant (1932) makes no mention of the occurrence of *E. siliqua*, although species living in a similar habitat, such as *E. ensis* and *Donax vittatus* (da Costa) are recorded. *Ensis siliqua* could hardly have been mistakenly identified as *E. ensis*.

Sea temperatures on either side of the Channel are given in Tables V and VI. The mean annual temperature at Plymouth and Jersey is almost the same, but the range of monthly means in Jersey is greater. At Roscoff the mean annual temperature is perhaps a little higher than at Plymouth, but the range of monthly means is much the same. Although the slightly higher summer temperatures on the southern side of the Channel may be important for the breeding of a species at the northern end of its range, such differences can hardly explain the differences in ecology of *Ensis* which ranges both north and south of the area. Another possible cause of the differences in ecology is discussed on p. 168.

TABLE V. MEAN MONTHLY AND ANNUAL SEA SURFACE TEMPERATURES FOR 1903–27 (° C.) AT THE NEAREST POSITIONS TO PLYMOUTH, JERSEY, AND ROSCOFF FOR WHICH SUCH DATA ARE AVAILABLE

(Data from Lumby, 1935.)

	50° 15′ N., 04° 15′ W. (Plymouth)	49° 08′ N., 02° 22′ W. (Jersey)	48° 52′ N., 04° 38′ W. (Roscoff)
Jan.	10·13	9·08	10·60
Feb.	8·70	8·06	9·83
Mar.	8·87	8·42	9·98
Apr.	9·43	9·29	9·74
May	10·96	11·45	11·10
June	12·88	13·75	12·43
July	15·16	15·59	14·72
Aug.	15·65	16·74	15·15
Sept.	15·02	16·27	14·22
Oct.	14·45	15·03	13·97
Nov.	12·76	12·86	12·90
Dec.	10·71	11·06	11·16
Mean annual	12·06	12·30	12·15

TABLE VI. MEAN MONTHLY AND ANNUAL SEA TEMPERATURES (° C.) AT PLYMOUTH AND JERSEY FOR THE YEARS 1947 TO 1951 INCLUSIVE

(Samples taken from the shore, daily. Data for Plymouth kindly supplied by G. H. Ivory and Partners, Plymouth; that for Jersey taken from the *Société Jersaise, Bulletin Annuel*, for 1948 to 1952.)

	Plymouth	Jersey
Jan.	9·0	6·9
Feb.	7·9	6·3
Mar.	8·3	7·3
Apr.	9·8	8·7
May	11·7	11·6
June	14·2	14·2
July	15·6	16·3
Aug.	16·6	17·6
Sept.	16·2	16·8
Oct.	14·7	14·6
Nov.	12·3	11·4
Dec.	10·2	8·9
Mean annual	12·2	11·7

NOTE ON THE LARVA OF *ENSIS*

All three species have a pelagic larva. Attempts at artificial fertilizations have not been entirely successful; adults collected during the spring of 1950 were never fully ripe, although Lebour (1938) states that *E. siliqua* breeds in early spring. A few larvae of each species were obtained, but only *E. ensis* was reared to metamorphosis. Shelled larvae, as described by Lebour, were obtained for all three species; these possess a large central cilium similar to that described by Lebour (1938, p. 139) for *Hiatella gallicana*, although Lebour states that no such cilium is present in *Ensis siliqua*.

Only a very few specimens of *E. ensis* were reared to metamorphosis, which took about 1 month at 13–15° C. The larvae were fed on Flagellate I, *Isochrysis galbana*. At metamorphosis these larvae had a shell length of *c*. 290μ. A late larva was seen swimming by its velum and occasionally extending its foot: at this stage it will presumably settle under suitable conditions.

Discussion

The factors believed to be of importance in the distribution of species of *Ensis* may be summarized as follows. *E. siliqua* and *E. ensis* are restricted to sands of a fairly fine grade in which the percentages both of silt and of coarse particles are fairly low. *E. ensis* shows a rather greater tolerance of silt and of coarse particles than *E. siliqua*. *E. arcuatus* inhabits sands of a coarser grade, but has a wide tolerance of different grades, so that its distribution overlaps that of the preceding species to some extent.

Sands black below the surface are avoided by all species.

E. siliqua can withstand a moderate degree of wave-exposure, but is absent from fully exposed beaches. *E. arcuatus* and *E. ensis* are restricted to more sheltered beaches. The stability of the soil is an important factor in determining the occurrence of all species on fairly exposed beaches.

On the shore, *Ensis* occurs only below mean low-water mark of spring tides. Offshore, all three species are common in shallow water. *E. siliqua* occurs down to only *c*. 20 m., but the other species occur in small numbers in deeper water on the Continental Shelf. Where wave action is so severe as to exclude a species from a particular beach, it may occur below low-tide marks where disturbance is less extreme.

South of the English Channel *E. siliqua* is much less common, while the tolerance of *E. arcuatus* and *E. ensis* to wave action appears to increase, so that they are found on moderately exposed beaches.

There is no evidence that the mineralogical nature of the sand grains, their shape or surface texture, currents, food supply or other biotic factors influence the absolute distribution of the species, although certain biotic factors may influence population density.

Ecological factors may limit the distribution of a species, either through a direct lethal effect, or by their influence on the choice of a particular environment. In the latter instance the adult may be living well within the lethal limits imposed by the environment, due to the effect of some form of 'habitat-selection', and as a result of which not all habitats in which the individual could survive become populated (cf. Elton, 1935, pp. 40–1). In sedentary marine organisms there is some evidence that the distribution of the adult is partly related to a choice by the larva of a suitable substratum in which to metamorphose.

Postponement of metamorphosis until a suitable substratum is reached has been recorded for a number of species (summarized by Wilson, 1952), including at least one mollusc, *Teredo*. Although larvae of several species have been shown to respond to the nature or particle size of the substratum, it is possible that others are induced to settle in the vicinity of adults or newly settled spat of the same species (cf. Knight-Jones, 1951). If the larva reacts to some feature of the environment other than the presence of other individuals of the same species, suitably devised settling experiments should explain some features of distribution. No such experiments have so far been made owing to difficulties in rearing the larvae. If, however, the larva reacts to the presence of other individuals of the same species, or if there is no highly specific settling reaction, the distribution of adults must be explained in terms of conditions favourable or otherwise to the individual after settlement.

The preference of species of *Ensis* for soils of a particular grade may well be due to a larval settlement reaction. The three species are so similar in structure and habits that it seems likely that any one species could burrow into and live in any of the full range of soils inhabited by all species. Silt, alone, may be a limiting factor to survival of the adult. The high silt percentages tolerated by *Ensis ensis* may be lethal to the other species. Pratt (1953) has shown that the lamellibranch *Venus mercenaria* grows more rapidly in sand than in a muddy sand, and this may be explained by the observations of Loosanoff & Tommers (1948), who found that even small quantities of suspended silt decrease the filtration rate of oysters (*Ostrea virginica*), which would adversely affect their food intake.

Reducing conditions in the soil, which are probably lethal to the adult, might affect distribution either at the settling stage or through subsequent mortality of newly settled individuals.

It is hard to see that *Ensis* larvae could distinguish the normal conditions of wave-exposure on a beach. Abortive colonization of more exposed beaches probably occurs, perhaps during calm weather, and when normal conditions of wave-action return the spat are washed out of the sand.

Ensis is probably restricted to low spring-tide level on the shore because it is not adapted to withstand the fluctuations of temperature, salinity, etc., that occur higher up the beach, but no explanation can be offered of the restricted depth range of *E. siliqua* offshore. Certain other species, e.g. *Donax vittatus* and *Tellina fabula*, have a similar depth range to *Ensis siliqua*.

Previous work on the ecology of benthic animals provides little information on the precise effects of particular limiting factors. The importance of soil grade has long been recognized (e.g. Allen, 1899; Ford, 1923; Davis, 1925; Prenant, 1932; Powell, 1937), but most workers have directed their attention to a classification of species or 'communities' in relation to soil grade rather than to the study of the occurrence of individual species in different localities, as has been attempted here. The *range* of substrata inhabited by different species is very variable, but one may expect members of the infauna to be more restricted in their range than members of the epifauna. Powell (1937) considers that deposit feeders are more restricted in their choice of bottom than herbivores and suspension feeders, while carnivores appear to show no particular preference. Holme (1949*a*) has shown that the silt content of the soil has an important effect on the distribution of certain species on the shore.

Properties of sands other than particle size may also affect distribution. Wilson (1953) has shown the importance of 'attractive' or 'repellent' substances, possibly of organic origin, on the surface of sand grains for the settlement of the larvae of the polychaete *Ophelia bicornis*. Ekman (1947) has correlated the distribution of certain invertebrates with the firmness of the soil, as measured by a cone penetrometer. Preliminary experiments by the writer with a small cone penetrometer attached to the end of a slender rod have not been entirely satisfactory. It is essential that measurements be made in the undisturbed substratum, and not, as was done by Ekman, in a dish in which the sample has been allowed to settle for 24 hr. Under such conditions the original packing is not regained.

On the effects of wave action on burrowing organisms, there are little but general observations. It is generally accepted that wave action excludes many species from exposed beaches, and the importance of this factor is emphasized by Southward (1953).

Davis (1923) has shown that water movements in the North Sea may influence the distribution of settling larvae of *Spisula*. Settlement of spat in limited areas will occur only where the adults are more or less confined to isolated patches. In *Ensis*, where the adults of all species are widely distributed in coastal areas, there should usually be sufficient numbers of larvae in any one place for all suitable substrata to be occupied. A possible exception may be the Scilly Isles, where only *E. arcuatus* has been found, although there are undoubtedly patches of sand of a suitable grade for the other species. A few miles from the shore the bottom slopes away to some 80 m. depth, so it is unlikely that *E. ensis* and *E. siliqua* occur in any numbers in the vicinity of the islands. The Scilly Isles are 23 miles from the Cornish coast, so that the density of larvae of the latter two species in the Scilly Isles plankton is probably small. Consequently small patches of soil of a suitable grade may be only occasionally colonized by these species. Similarly, the relative scarcity of coarse-grade deposits in Great West Bay will result in a scarcity of breeding stock of *E. arcuatus*, so that suitable deposits in the area may not always be colonized.

Detailed observations have been confined to an area bordering the western half of the English Channel. The apparent differences in distribution on either side of the Channel makes one chary of applying conclusions to other areas, however. Temperature differences seem to be insufficient to account for the changes in distribution (pp. 163–4). There is probably little interchange between populations on either side of the Channel, so it is possible that races with different habitat-requirements occur. The western end of the English Channel is known to be an important boundary to the distribution of certain species (Ekman, 1953, pp. 81–2), and this boundary may be as much due to the geographical isolation of shallow-water species on each side as to temperature differences. Rees (1950) has shown that larvae of *Octopus vulgaris*, which have a pelagic life of at least a month, can spread to the south coast of England from breeding centres in the Channel Islands region, and one can presume that a similar interchange of *Ensis* populations does occur from time to time. Such interchange is probably on a restricted scale, and it is possible that different races could survive in spite of it.

SUMMARY

An attempt is made to analyse the ecological factors affecting the distribution of the three European species of *Ensis*: *E. siliqua* (L.), *E. ensis* (L.) and *E. arcuatus* (Jeffreys).

All three species appear to be confined to European waters, but the exact limits of their range have not been established.

None of these species appears to occur outside Europe, but the North American *E. minor* Dall may be the same as *E. ensis*.

Soil grade is shown to be an important factor limiting distribution, although no species is narrowly confined to soil of a particular grade composition. Population densities are in general higher in soils approaching the 'average' grade inhabited by each species.

There is no evidence that the shape or mineralogical composition of the sand grains affects distribution.

All species avoid black, reducing, sands.

All species require some shelter from wave action, but *E. siliqua* can withstand much greater wave exposure than the other two. Tolerance of wave-action seems to depend on the stability of the beach deposits.

The three species are found at LWST on the beach, and in shallow water offshore. *E. siliqua* has a restricted depth range of *c.* 20 m., but the other species may be found in small numbers in deeper water.

Distribution in Jersey and Finistère differs from that in south-west England in greater tolerance of wave exposure by *E. arcuatus* and *E. ensis*.

Each species has a pelagic larva. *E. ensis* larvae were reared to metamorphosis in about a month.

The mode of action of limiting factors is discussed, and the results are considered in the light of previous work on the ecology of benthic invertebrates.

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APPENDIX

TABLE VII. LIST OF LOCALITIES

The name of the locality is followed by the serial number of the soil sample, enabling cross-reference to Tables I–III to be made. The occurrence and density of the species is shown next, in brackets: A, *Ensis siliqua*; B, *E. arcuatus*; C, *E. ensis*. This is followed by a National Grid Reference to the station, or its latitude and longitude. The depth of water at offshore stations is also given.

Shore Collections

DEVON

Polesands, Exmouth. S.W. corner. 47 (A, scarce). 20/998792.
Dawlish. Mid-way between Dawlish and Langstone Pt. 74 (A, occasional). 20/971773.
Torr Abbey Sands. 139 (A, common), 140 (C, scarce), 141 (A, common). 20/912635.
Paignton beach, S. end. 10 (A, common). 20/896605.
Goodrington Sands. N. end of beach. 60 (A, common). 20/895598.
  Middle of S. half of beach. 59 (A, common). 20/895593.
Saltern Cove. 53 (A, scarce). 20/896585.
Broadsands. N. end of beach. 11 (A, common; C, occasional). 20/897578.
  Middle of beach. 2 (A, common; C, occasional). 20/897576.
Elbury Cove. 105 (C, scarce), 106 (A, common; C, scarce). 20/903570.
Salcombe, E. S. side of Millbay. 6 (A, occasional; B, occasional). 20/740382.
  N. side of Millbay. 3 (B, common), 12 (B, common; C, occasional), 63 (A, scarce). 20/740383.

Salcombe, W. 200 yd. S. of Marine Hotel. 64 (B, scarce). 20/738385.
Woodville Rocks. 65 (B, scarce), 163 (C, scarce). 20/736384.
Yealm River. Cellars beach. 70 (B, occasional), 71 (B, occasional), 72 (C, scarce). 20/531476.
Below Passage Wood. 69 (B, scarce). 20/537476.
Yealm sand bank. 1 (B, common). 20/539481.
Thorn Pt. 171 (B, scarce). 20/542490.
Drake's Island. E. end of N. side. 172 (A, scarce; C, scarce). 20/470529.
E. side. 173 (B, occasional). 20/471528.
E. of pier. 174 (C, scarce), 176 (A, scarce). 20/469529.
W. of pier. 175 (A, scarce; B, occasional; C, occasional). 20/468529.

CORNWALL
Whitsand Bay, Sharrow Pt. 117 (A, scarce). 20/393521.
Par Sands. E. end of beach. 68 (A, common). 20/087524.
Nr. Callyvador rocks. 66 (A, occasional). 20/083524.
Duporth. 62 (A, common). 20/036511.
Porthpean. 61 (A, scarce). 20/033507.
Pentewan. 136 (A, occasional). 20/019464.
Helford River, Gate Beach. 24 (C, occasional). 10/760268; 36 (C, occasional), 38 (A, occasional), 40 (C, occasional), 41 (A, scarce; C, occasional), 164 (B, scarce), 165 (A, occasional; B, scarce; C, occasional). 10/762268.
Flushing Cove (Gillan harbour). 56 (B, scarce). 10/787252. 54 (C, scarce), 55 (A, common; C, occasional), 57 (B, scarce), 58 (A, scarce), 162 (C, occasional). 10/784252.
Marazion. 52 (A, common). 10/509309.

SCILLY ISLES
Tresco, E. of Rushy Pt. 50 (B, common). 00/903151.
Tresco-Bryher channel. 120 (B, common), 121 (B, common). 00/886145. 122 (B, common), 00/886144. 130 (B, common). 00/884149. 131 (B, common). 00/884152.
Foreman's Island. 126 (B, common). 00/901158. 127 (B, common). 00/901160. 128 (B, occasional). 00/900161. 129 (B, common). 00/898162.

AYR
Fairlie Sands. 18 (A, common). 26/196541.

NORTHUMBERLAND
Newton Haven. 19 (B, common). 46/247241.

PEMBROKE
Dale Flats. 25 (A, occasional), 12/813058. 73 (A, common). 12/814060.

CHANNEL ISLANDS
Grouville Bay. 7 (C, scarce), 9 (B, common), 16 (B, occasional; C, scarce). 49° 10′ 25″ N., 02° 00′ 45″ W.
St Aubin's Bay. 15 (B, scarce), 21 (C, common). 49° 11′ 00″ N., 02° 09′ 35″ W.

FINISTÈRE
Penpoull. 145 (C, occasional). 48° 41′ 42″ N., 03° 57′ 00″ W.
Térénès. 147 (C, scarce), 149 (B, scarce; C, scarce). 48° 40′ 45″ N., 03° 51′ 12″ W.
Grève de S. Michel. 155 (A, occasional). 48° 41′ 15″ N., 03° 36′ 00″ W.
Grève de Goulven. 156 (C, scarce), 157 (B, occasional), 158 (B, common). 48° 39′ 40″ N., 04° 15′ 00″ W.
Perharidy. 159 (B, occasional). 48° 43′ 55″ N., 04° 00′ 06″ W.

TABLE VII (*cont.*)

Grab and Dredge Samples

Great West Bay. Station 12. 27 (C, occasional). 50° 35′ 12″ N., 03° 26′ 25″ W. 5·5 m.
Station 16. 29 (C, occasional). 50° 34′ 40″ N., 03° 26′ 48″ W. 10·5 m.
Station 17. 28 (C, occasional). 50° 34′ 25″ N., 03° 27′ 05″ W. 10·5 m.
Station 19. 30 (C, common). 50° 33′ 10″ N., 03° 27′ 00″ W. 16·5 m.
Torbay. Station 9. 167 (A, common; C, occasional). 50° 24′ 30″ N., 03° 32′ 54″ W. 3·2 m.
Station 18. 169 (A, occasional). 50° 25′ 24″ N., 03° 33′ 12″ W. 6·1 m.
Station X. 170 (C, occasional). 50° 24′ 12″ N., 03° 32′ 36″ W. *c.* 5·0 m.
Plymouth. 22 (A, common). 50° 15′ 45″ N., 03° 53′ 00″ W. 20 m.
116 (C, scarce). 50° 10′ 00″ N., 04° 10′ 00″ W. 60 m.
113 (C, scarce). 50° 15′ 00″ N., 04° 12′ 30″ W. 49 m.
32 (C, scarce). 50° 11′ 00″ N., 04° 05′ 00″ W. 58·5 m.
31 (B, scarce; C, occasional). 50° 19′ 42″ N., 04° 14′ 45″ W. 14·5 m.
St Austell Bay. 33 (C, occasional). 50° 17′ 15″ N., 04° 46′ 18″ W. 9 m.
34 (C, occasional). 50° 17′ 24″ N., 04° 45′ 42″ W. 13 m.
35 (C, occasional). 50° 19′ 30″ N., 04° 45′ 06″ W. 6 m.
Aberporth Bay. 160 (C, common). 52° 08′ 48″ N., 04° 31′ 48″ W. 13 m.
Irish Sea. 14 (C, occasional). *Approx.* 54° 23′ N., 03° 35′ W.

'Negative' Records

Records of sandy beaches on which no *Ensis* was found. Where the beach was examined on a very low spring tide, the name is in italics. The probable reasons for absence of *Ensis* are given thus: R, black, reducing sands; S, shifting, unstable sands; E, wave action extreme; L, lowered salinity owing to fresh water running over the beach at low tide. *, beaches where *Ensis* may have been washed up from deeper water, most of the beach being barren.

DEVON
Exmouth, nr. Maer Rocks. R. (30/010798.)
Polesands.* S. (30/002794.)
Salcombe, S. of Millbay. S. (20/737379.)
North Sands. L? (20/731381.)
South Sands. L? (20/729377.)
Borough Island and Challaborough Bay. E. (20/655439, 648443, 648448.)
Mothecombe. R., L? (20/612471.)
Bovisand. R. (20/492506.)
Saunton Sands (N. Devon). E. (21/435345–438378.)

CORNWALL
Whitsand Bay.* E. (*ca.* 20/407510.)
Charlestown. L? (20/040514.)
St Michael's Mount. R. (10/515303.)

SCILLY ISLES
Bryher, two beaches at S.W. end of island. E. (00/873141, 876140.)

JERSEY
St Aubin's Bay (middle). E. (49° 11′ 18″ N., 02° 08′ 42″ W.)

FINISTÈRE
Grève de Goulven (E. end). E. (48° 39′ 48″ N., 04° 14′ 00″ W.)
South of Isle de Sieck. E? (48° 42′ 00″ N., 04° 04′ 30″ W.)

J. Mar. biol. Ass. U.K. (1954) 33, 173–175
Printed in Great Britain

EFFECT OF EXTERNAL MILIEU ON LUMINESCENCE IN *CHAETOPTERUS*

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In a previous paper some effects of anisosmotic and unbalanced salt solutions on the luminescent responses of *Chaetopterus variopedatus* have been described (Nicol, 1952). Similar experiments have been carried out on polynoid elytra, and the results are described elsewhere in this Journal, together with a brief review of the relevant scientific literature (Nicol, 1954*b*). The present experiments were undertaken to investigate the effects of single salts in physiological concentrations, balanced by the addition of some inert substance, and to explain the possible mechanism of stimulation by hypo-osmotic solutions.

The experiments were simple. Whole specimens of *Chaetopterus*, or isolated anterior regions, were placed in the solutions to be tested. Salts ($NaCl$, KCl, $CaCl_2$, $MgCl_2$), sucrose and choline chloride were made up in solutions osmotically equivalent to normal sea water. Alkalinity of salt solutions was raised to pH 8·2.

Results were as follows:

Choline Cl	No luminescent response
Sucrose	Faint light appeared slowly
NaCl 81 + choline Cl 19	Faint brief light
KCl 2 + choline Cl 98	Faint brief light
$CaCl_2$ 3 + choline Cl 97	Little faint light, especially when container agitated
NaCl 81 + sucrose 19	Luminescent response
KCl 2 + sucrose 98	Luminescent response
$CaCl_2$ 3 + sucrose 97	Luminescence fairly bright
$MgCl_2$ 15 + sucrose 85	No light
NaCl 81 + fresh water 19	Luminescent response
KCl 2 + fresh water 98	Faint, brief light
$CaCl_2$ 3 + fresh water 97	Luminescent response
$MgCl_2$ 15 + fresh water 85	Luminescent response

Specimens were anaesthetized for half an hour in 0·2 % chloretone or 0·5 % cocaine (made up in sea water). They were then transferred to sucrose containing the same quantity of anaesthetic. A feeble luminescent response resulted.

These results confirm those obtained in earlier experiments, namely that solutions of the single salts NaCl and KCl are excitatory and evoke luminescence; $CaCl_2$ raises irritability, resulting in faint light, especially if the preparation is subjected to gentle mechanical stimulation. In addition, the results show that these three salts produce their effects not only when acting in excess, but also when, balanced with choline chloride, they occur in normal physiological amounts.

Contrary to the effect of choline chloride, which appears to be physiologically inert so far as the luminescent response is concerned, sucrose evokes a faint luminescent response which is not blocked by anaesthesia with chloretone or cocaine, but which does not appear in the presence of $MgCl_2$.

As previously noted, hypo-osmotic sea water (25%) evokes luminescence, and luminescence appears in solutions of each of the four ions, Na, K, Ca and Mg, in physiological concentration plus fresh water. It is noteworthy that neither Ca nor Mg, in physiological quantity, block hypo-osmotic excitatory stimulation.

In an investigation of the viability of various marine animals in diluted sea water, Pearse (1928) found that *Chaetopterus* would tolerate 50% sea water for long periods. A previous study has shown that luminescence does not appear until the sea water is diluted 50% or more (Nicol, 1952). This is the physiological limit of the species, and the harmful effects of dilutions greater than 50% are not offset by $CaCl_2$ and $MgCl_2$ in physiological quantities.

In any system consisting of excitable tissue dependent upon a properly balanced ionic environment for stability, any radical alteration in the concentrations or proportions of the external ions will produce great changes in excitability, and depolarization of external boundaries. Changing the external milieu could affect either the nervous system or the luminescent cells. Evidence has recently been adduced to show that the latter possess a contractile mechanism for discharging the luminescent secretion. The excitatory effect of Na and K on excitable tissues is well known, and these two ions probably act on both the photogenic cells and nervous system of *Chaetopterus*. The responses which they induce are by no means as bright or as long-lasting as those evoked by strong electrical stimulation, probably owing to initial localized depolarization, followed by loss of excitability as this process becomes complete. The increased irritability produced by Ca has been reported for other invertebrate groups, and may be linked with Mg-lack (Robertson, 1941; Nicol, 1954*a*, *b*).

As in the vertebrate preparation, choline chloride appears to be inert; any possible blocking action such as it exerts on the neuro-muscular junction has not been ascertained. Sucrose, on the other hand, has a weak stimulatory effect in isosmotic concentration. Sucrose, therefore, is osmotically but not ionically inert. Its stimulatory effect on the luminescent response is not abolished by prolonged nervous anaesthesia, and it probably acts directly on the photogenic cells apart from any effect on the nervous system. The ionic unbalance produced by the introduction of isosmotic sucrose produces some degree of depolarization owing to ionic shifts, and results in excitation and luminescence. The same effect is probably responsible for the luminescence appearing in diluted sea water or fresh water.

In a recent paper Bonhomme (1953) gives a histological picture of luminescent secretion in *Polycirrus* rather similar to that in *Chaetopterus*. The course of secretion is presented as opening of the cell, hydrolysis of secretory granules, and expulsion of photogenic material, possibly as the result of swelling or changed osmotic relations. The possibility that cytolysis or raised intracellular pressure resulting from imbibition of water may be responsible for the luminescence of *Chaetopterus* in dilute media has been considered, and seems most unlikely in view of the faint and transitory light produced, compared with the brighter response which can be evoked by electrical stimulation, and with the amount of photogenic material present. Dilute sea water and fresh water probably exert a transitory depolarizing effect on the excitable tissues, which rapidly lose irritability. A similar explanation is probably applicable to the many instances of luminescence in marine animals evoked by application of fresh water.

SUMMARY

The effects of hypo-osmotic solutions, and of isosmotic solutions of choline chloride, sucrose, NaCl, KCl, $CaCl_2$ and $MgCl_2$ on the luminescent response of *Chaetopterus variopedatus* are reported. Choline chloride, as an inert substance, can be used in conjunction with each of the other salts to test the effects of the latter in physiological quantities. Sodium and potassium excite in physiological concentrations; calcium raises irritability. Sucrose produces a weak luminescent response, which is not blocked by nervous anaesthetics (cocaine, choloretone). Changes in the ionic environment induce localized depolarization, followed by complete depolarization and loss of irritability of both nervous and photogenic tissue. This explanation will probably account for most instances of luminescence evoked by ionically abnormal media.

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J. Mar. biol. Ass. U.K. (1954) 33, 177–186
Printed in Great Britain

FATIGUE OF THE LUMINESCENT RESPONSE OF *CHAETOPTERUS*

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(Text-figs. 1–6)

When stimulated in some suitable manner *Chaetopterus* secretes a luminescent material into the surrounding sea water. The luminescent secretion is discharged from glands which are widely dispersed over the surface of the animal; the most conspicuous are two glandular patches on the dorsal surface of segment XII. Secretory material is forced out of the cells by some contractile process; discharge is not merely the result of secretion pressure. Under repetitive stimulation the intensity of the luminescent response decreases owing to the intervention of fatigue. Fatigue has been interpreted as a gradual exhaustion of luminescent material in the glandular cells (Nicol, 1952*b*, *c*). The present investigation seeks to analyse the onset and progress of fatigue in greater detail.

The cost of part of the equipment used in this research was met by a grant-in-aid of scientific research from the Royal Society. I acknowledge with gratitude technical assistance from Mr A. E. Stoate and Mr F. J. Warren.

MATERIAL AND METHODS

Fresh specimens of *C. variopedatus* were employed. These were stimulated by brief condenser shocks from an electronic or mechanically operated circuit. The luminescent response was recorded by means of photomultiplier tube (RCA 931), direct coupled amplifier, and oscilloscope. Deflexions of the spot on the oscilloscope screen were photographed with moving paper.

RESULTS

Diminution of Response under Repetitive Stimulation

Specimens were stimulated electrically for different periods and at different frequencies. When subjected to a series of shocks, with long intervals between stimuli, the response takes the form of separate flashes. Fig. 1 shows a series of records obtained by administering an electrical shock every 10 min. Each shock evokes a luminescent response, which quickly rises to a peak, and gradually decays over the course of 5 min. The intensity of consecutive flashes is plotted in Fig. 2. The first few responses are of about equal magnitude, but thereafter intensity rapidly decreases. The decay curve approaches a quarter ellipse in form. At this very slow rate of stimulation, 1 per 10 min., it is reasonable to assume that no fatigue of the contractile mechanism is occurring, and that diminution of the response is due to intervention of other factors.

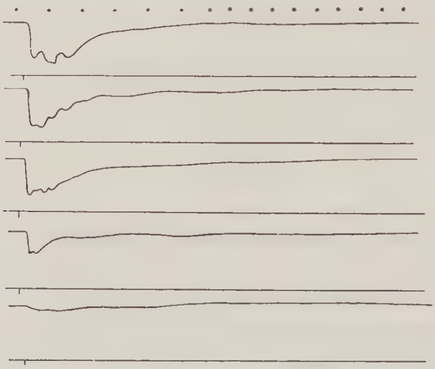

Fig. 1. Fatigue of the luminescent glands of *Chaetopterus* (glands on segment XII). Five consecutive records from the same animal, responses separated by an interval of 10 min. Stimulus on each occasion was a single condenser shock, shown as a pip on the lower line. Luminescent response appears in these records as a deflexion downwards of the middle trace. Time scale above, 1 per 30 sec. Paper speed halved after 2½ min.

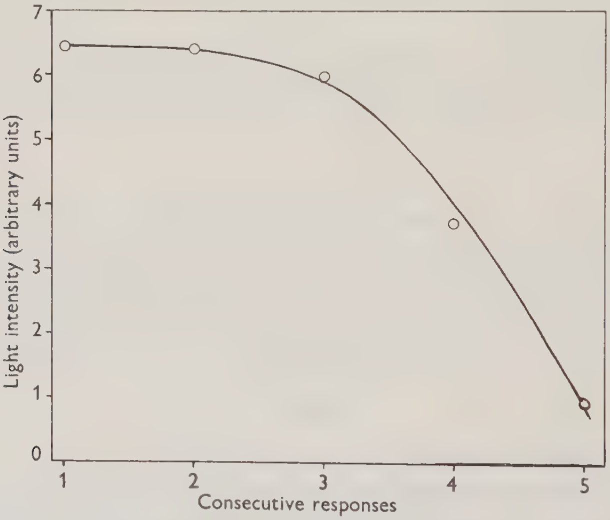

Fig. 2. Curve showing decrease of intensity of consecutive luminescent responses. Each response evoked by a single stimulus.

A burst of stimuli at slow rates (2–6 per min.) evokes recognizably discrete responses which summate, especially at higher frequencies (Fig. 3). When stimulation is maintained at these frequencies for some time, the response (light intensity) gradually falls off. With rest periods of 5 min. between successive bursts of stimuli, each successive period of stimulation evokes a luminescent response which is progressively less than that of its predecessor (Figs. 3B, 4A). A point of some importance for the argument which will be

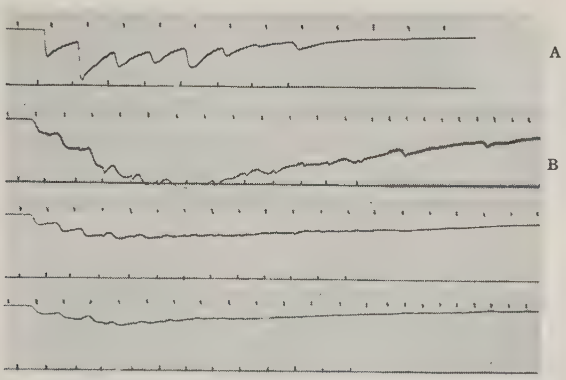

Fig. 3. A, luminescent responses evoked by a burst of stimuli (8 stimuli at 2 per min.) for 4 min. Time scale, 1 per 30 sec. B, responses to repetitive stimulation. Three consecutive periods of stimulation at 6 per min. for 2 min. Intervals of 5 min. between periods of stimulation. Time scale, 1 per 10 sec.

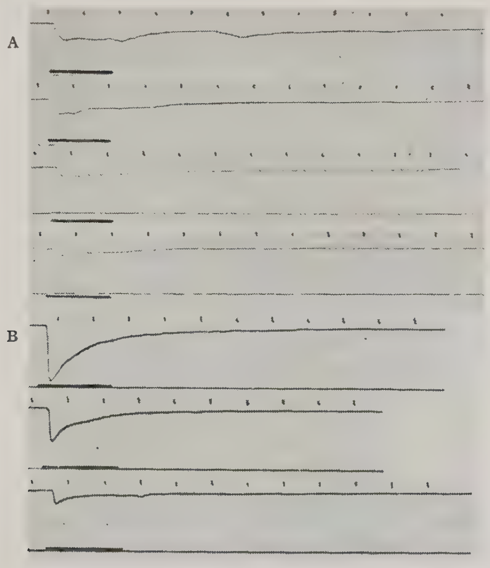

Fig. 4. A, consecutive responses to repetitive stimulation. Each response was evoked by a 50 sec. burst at 1 per sec. Interval of 5 min. between successive periods of stimulation. Duration of stimulation is shown in heavy black on lower line of each record. Time scale, 2 per min. B, responses to repetitive stimulation. Experimental conditions and details of recording similar to above, except that a frequency of 2 per sec. was used.

developed is that the maximal intensity developed in each response (after the first) is about equal to that developed at the termination of the previous period of stimulation and, in any event, is no greater. At a stimulation frequency of 1 per sec., the ratio of maximal light intensity at the beginning of a response to intensity at the end of the previous period of stimulation is unity.

With prolonged stimulation at faster rates, above 1 per sec., another phenomenon becomes apparent, viz. fatigue of the secretory mechanism. This is brought out in Figs. 4B and 5A (50 sec. bursts at 2 and 5 per sec.). The records show a gradual diminution of light intensity during each successive period of stimulation. But they also reveal that during the course of a prolonged burst at higher frequencies, the response tends to fall off rapidly.

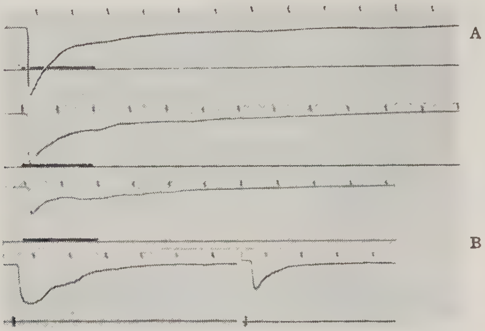

Fig. 5. A, responses to bursts of repetitive stimulation. Each response was evoked by a 50 sec. burst at 5 per sec. Consecutive responses of the same animal; 5 min. interval between each period of stimulation. B, left, response produced by 2 stimuli (interval between shocks 0·5 sec.). Right, same specimen, response evoked by a single shock. Time scale, 2 per min.

Moreover, the maximal intensity developed in each successive response is greater than the light intensity obtaining at the termination of the previous period of fast stimulation. At a frequency of 2 per sec. (50 sec. burst), ratios of initial luminescence to luminescence at termination of the previous period of stimulation are 2·0–2·5. In this experiment there were rest periods of 5 min. or more between successive bursts of stimuli.

Summation

Summation of consecutive luminescent responses has been demonstrated in previous experiments (Nicol, 1952*c*). At slow frequencies, from 2 to 12 per min., separate responses are often, but not always, additive, and light intensity may rise during progress of stimulation (Fig. 3). Fusion of discrete responses occurs above 30 per min. An attempt made previously to demonstrate facilitation in the luminescent response was unsuccessful, and fresh experiments were devised in which the nerve cord was stimulated by strong shocks, instead of direct stimulation of the luminescent glands. Comparison was made of 1 versus 2 condenser shocks; in the latter event the two stimuli were separated by an interval of 0·5 sec. As previously noted, the response following two stimuli is sometimes equal to, or less than, that resulting from a single stimulus (Nicol, 1952*c*). But with the present technique some records were obtained (two out of five) in which the response produced by a pair of stimuli was significantly greater than that evoked by a single stimulus (Fig. 5B). Magnitude of response (maximal intensity, total light) produced by two stimuli was not more than twice that produced by a single shock. This appears to preclude facilitation and to demonstrate that increment of response, under repetitive stimulation, is due to summation.

Recovery from Fatigue

Earlier attempts to demonstrate recovery of the luminescent response after fatigue were unsuccessful. A few observations suggest that a rest of several hours following stimulation permits recovery of the luminescent response (Nicol, 1952*b*). Recovery of luminescence has been investigated in the following manner. Whole specimens were stimulated with a prolonged burst of shocks at slow frequencies, and records were obtained of the response. Electrodes were placed either directly on the glandular cells, or over the ventral nerve cord of segment XII. With electrodes in the latter position stimulation invariably caused autotomy of the anterior region as well, and these preparations were used only to investigate recovery over short periods not exceeding 24 hr. After stimulation the specimens were returned to sea water. Those animals to be examined in 24 hr. or later were placed in empty *Chaetopterus* tubes. Whole animals soon demonstrated normal activity by fabricating new sections on cut ends of the tubes. Thirty-three animals were investigated.

It was found that specimens varied considerably in time taken to achieve complete recovery. After periods of $4\frac{1}{2}$, 24 and 48 hr., animals displayed 10–100% of the luminescent response (original response rated at 100%) (Fig. 6). After 72 hr. all specimens which were examined gave a luminescent response equal to or greater than that originally recorded. Complete recovery of the luminescent response, following exhaustive stimulation, therefore, can take place within 5 hr.

HISTOLOGICAL OBSERVATIONS

The histology of the luminescent glands of *Chaetopterus* is reviewed in an earlier paper (Nicol, 1952*a*). On the dorsal surface of segment XII the glands consist of dense aggregations of elongated cells packed with secretory granules The secretory contents form a bag-shaped mass invested by a cytoplasmic sheath, the cell wall. The small nucleus lies at the base of the cell, from which fibres run to the basement membrane. The cell walls are strongly argentophilic, suggesting a dense groundwork, but no structural differentiation is apparent. Interspersed between the glandular cells are numerous ciliated cells. These cells have been carefully examined in preparations stained with iron haematoxylin or treated with silver protargol. It is found that the ciliated cells are very slender elongated elements lying between the luminescent cells. Distally the ciliated cells flare out into trumpet-shaped expansions which partially cover the external region of the luminescent cells. They bear long cilia on their external surfaces; the cilia arise in the usual way from basal granules, from which a cone of fibres can be followed a short distance into the cell towards the nucleus.

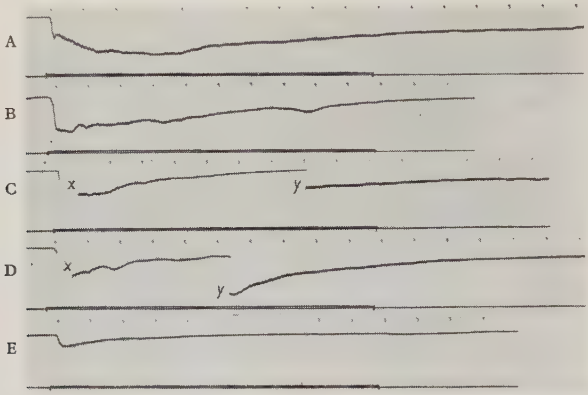

Fig. 6. Fatigue of luminescent response and recovery from fatigue. Stimulation in each record, 5 min. burst at 30 per min. Time scale, 1 per 30 sec. A, first response; B, second response from the same specimen after an interval of 4½ hr., C, first response from another specimen; D, second response from the same after an interval of 24 hr.; E, another response, 3 min. after D. At *x*, amplification was reduced to one-fifth and increased again at *y*.

Ciliated cells in the intestine of *Lumbricus* are said to possess contractile intracellular fibrils which assist in the extrusion of the secretory material from contiguous glandular cells by exerting compression on the latter (Millott, 1948). The appearance of these cells is not dissimilar to that seen in the luminescent glands of *Chaetopterus*, except that I have not been able to satisfy myself that any system of strongly developed intracellular fibrils extends through the entire length of the ciliated cell. The appearance of the luminescent cells when secreting strongly suggests that the glandular contents are being forcibly expelled, presumably by contraction of the cytoplasmic sheath of the glandular cell, which may contain contractile protein. Secreting cells differ in appearance from quiescent cells in that the glandular contents of the former are pushed towards the free surface of the cell while the base of the cell is compressed and tapering. The ciliated cells serve to drive the discharged luminescent material towards a ciliated tract on the median dorsal surface, in which it is carried anteriorly, and dispersed away from the animal.

DISCUSSION AND CONCLUSIONS

The data which have been secured in this investigation can be summarized as follows. A single stimulus evokes a bright response, and repetitive stimuli result in summation of luminescent responses. There is no evidence for facilitation in the neuro-effector system. Repetitive stimulation at slow rates (below 1 per sec.) brings about gradual exhaustion of the luminescent response, as evidenced by the effect of further stimulation after a short period of rest. Complete recovery of luminescent ability is achieved in 4–5 hr. in some specimens, longer in others. Under repetitive stimulation at fast rates, above 1 per sec., the response quickly decays during the course of stimulation, but recovers after a brief interval of 5 min. so that the maximal intensity developed during the second period of stimulation is greater than that measured towards the end of the previous period of stimulation. Histological examination of the luminescent glands reveals that much secretion is still present in the glandular cells, even after exhaustive stimulation. The following hypothetical interpretation is offered to account for these facts.

Prolonged stimulation at high frequencies (above 1 per sec.) probably brings about gradual fatigue of the contractile mechanism responsible for extruding the luminescent material. Consequently, the cell is no longer able to maintain its contraction, and extrusion of luminescent material declines. A rest period of 5 min. is sufficient to allow recovery of contractile efficiency, and to permit another maximal response. The correspondence to muscle fatigue is obvious. Curves of tetanic contraction recorded from the longitudinal muscle of the sabellid *Branchiomma*, for example, show a similar sequence of events, i.e. rapid decline of tension and contraction-height during the course of stimulation, and recovery of contractile ability after a period of rest, so that the initial tension developed by the rested muscle is greater than the tension developed during the terminal period of stimulation in the previous contraction (Nicol, 1951). This similarity, of course, sheds no light on the physico-chemical events responsible for fatigue, but it emphasizes that the same sort of contractile mechanism is operating in the discharge of luminescent material as in muscular contraction.

In the absence of any other apparent mechanism it is suggested that contraction is accomplished by the cytoplasmic sheath of the glandular cells. These may be endowed with contractile proteins capable of exerting pressure and producing movement.

The progressive diminution of light intensity which occurs in successive discrete responses is due to factors other than contraction-fatigue. Part of this decrease must result from progressive exhaustion of luminescent material available for discharge. At first sight this would seem to provide an adequate explanation for the gradual decline in brightness of successive responses. There are several reasons for considering this explanation inadequate. First, if glands are electrically stimulated to exhaustion until the luminescent response beomes very weak, and are then mechanically disrupted, a great deal more light is produced. Secondly, if glands electrically stimulated to exhaustion are examined histologically, it becomes evident that a large amount of secretory material is still present inside the cells. It is evident, therefore, that some other factor is operating which is not merely fatigue of the contractile mechanism, causing decreased efficiency of contraction during prolonged activity, and resulting in conservation of luminescent material intracellularly.

It is noteworthy that the curve showing progressive diminution of light intensity in successive isolated responses has the form roughly of a quarter ellipse. That is, the first few responses (2 or 3) maintain a high intensity, after which maximal light intensities fall off at an increasing rate. Similar curves showing decrease of intensity in consecutive luminescent responses of polynoids take the form of rectangular hyperbolas. That is, the intensities of luminescent responses after the first one fall off at a decreasing rate along an asymptotic curve. In these latter animals the controlling factor is probably progressive exhaustion of intracellular luminescent material, or some other substance controlling oxidation of the latter.

Any suggestions concerning the factor responsible for this apparent fatigue of luminescent ability which develops progressively in isolated consecutive responses of *Chaetopterus* must be speculative, with the indirect information at hand. Attention should be directed first of all to the saccular organization of the glandular cells. Once the cell starts to secrete a distal pore opens on the cell surface, and secretory granules are forced out. A fresh unstimulated glandular cell will be distended and its wall stretched. When stimulated it will compress the cell contents and the pressure will find relief on extrusion of some of the cellular contents. A small proportion of the intracellular secretory material is lost with each contraction. A second stimulus and contraction will find the cell less distended than on the first occasion. From this viewpoint the glandular cell can be regarded as a semi-fluid hydraulic system in which pressure exerted on an enclosed column leads to movement of the latter and compensatory pressure-release once the cell opens to the outside. An analogy is at hand in the fluid-filled cavities of soft-bodied animals such as annelids. But in these forms the fluid-volume remains constant, and provides a hydrostatic system against which the muscles can operate. The contents of the glandular cell, however, are gradually dissipated during successive responses. On the assumption that the contractile mechanism of the cell behaves like muscle under stretch, it may be expected to produce maximal compression when stretched or expanded, and the force which it develops will fall off as it progressively diminishes in pace with reduction of cellular contents and cell-size. The curve in Fig. 2 showing progressive diminution in light intensity of successive responses is very similar to a curve for striated muscle when tension developed is plotted against decreasing length along the x-axis.

It is impossible from these data, and probably impossible from experiments designed in this way, to obtain a true picture of the course of recovery and restitution of secretory material in the luminescent cells. Even after an exhaustive stimulation of the luminescent glands the cells show no visible decrease of secretory material, and the problem cannot be resolved by histological means. The luminescent response is regenerated to full intensity in some 4–5 hr., even in isolated heads severed from the rest of the body. This possibly could be due to restitution of glandular material. It could also result from temporary intake of sea water, restoring cell volume.

This hypothesis and these analogies are presented for argument. As a tentative hypothesis it has this to commend it. It provides an explanation of the progressive decrease in intensity of successive responses. It accounts for progressive exhaustion of the secreting (contractile) mechanism in the face of high residual levels of intracellular luminescent material. And it evokes no new principles not already described for contractile tissues.

SUMMARY

Further experiments have been carried out on the luminescent glands of *Chaetopterus* in an attempt to provide a functional explanation of the mechanism of glandular secretion. Attention has been confined to the luminescent glands in segment XII, which were excited by electrical stimulation. Responses have been followed and recorded by photomultiplier tube and oscilloscope.

A bright response is evoked by a single stimulus, and repetitive stimulation at rates slow enough to allow separate responses to be distinguishable produces summation. Paired stimuli provide no evidence for facilitation. The intensity of the response produced by two stimuli is not more than twice that produced by one.

Evidence is adduced for the participation of three factors in fatigue of the luminescent response. At rapid rates of stimulation the contractile mechanism responsible for extruding the secretory material becomes fatigued. At very slow rates of stimulation, below that producing fatigue of contractility, light-intensity falls off in consecutive responses. This is only in small part due to gradual exhaustion of available luminescent material, since the glandular cells still contain large amounts of secretion even after stimulation to apparent functional exhaustion. The hypothesis is advanced that the contractile mechanism exerts maximal contraction when fully stretched in a cell heavily loaded with secretory material, and its efficiency decreases as it shrinks with reduction of cell volume.

Complete recovery of luminescent ability, to intensities initially recorded, was attained by some specimens in 5 hr.; all specimens examined showed complete recovery in 72 hr.

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J. Mar. biol. Ass. U.K. (1954) 33, 187–224
Printed in Great Britain

THE CREVICE FAUNAS OF THE UPPER INTERTIDAL ZONE AT WEMBURY

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(Text-figs. 1–7)

The main picture of the zonation of life between tide marks on British shores has been added to by a good deal of recent work, such as that of Colman (1932) and Evans (1947) at Plymouth, and the regional studies by Stephenson & Stephenson (1949). Of the more restricted special habitats within the tidal area there have been fewer accounts, though Colman (1940) has made a detailed survey of the faunas inhabiting intertidal seaweeds, which was later followed by Wieser (1952). Most recently there has appeared a paper by Glynne-Williams & Hobart (1952), working at Anglesey, which for the first time analysed clearly the composition and food relations of the restricted fauna living in crevices in this habitat. This part of the tidal zone forms an interesting meeting place of two faunal elements, those intertidal animals of terrestrial origin and those which are truly marine. In the summer of 1950 and 1951 the present writer had made a similar study in the Plymouth area, at Wembury and some other localities. The work was begun as part of an ecological study of the two marine pulmonates *Leucophytia bidentata* and *Otina otis*, and was later extended to take account of the other animals hidden in crevices and those living on the exposed rock surface throughout the upper half of the tidal zone.

The work of Glynne-Williams & Hobart lends a further interest to the Devon results as a comparative study, and—while in many respects both this and the Anglesey work must serve as preliminary studies only—the following account may be of value (i) in making a comparison with the larger and well-developed crevices in another type of foliaceous rock, (ii) in giving some quantitative data on the vertical and horizontal distribution of animals in crevices, (iii) in showing the differences in detail between the essentially similar crevice faunas in the two localities, and (iv) in making a comparison between conditions applying to ecological succession in crevices with conditions of life on the open rock surface.

The seaweed-inhabiting faunas are not further discussed here, with the single exception of the lichen *Pygmaea pumila* (= *Lichina pygmaea*). The observations of Glynne-Williams & Hobart on the origin, and the food and moisture content of the deposits present in crevices are also taken as broadly applicable to Devon conditions, and here again the present writer has no new contribution to make.

This work was carried out at the Plymouth Laboratory of the Marine Biological Association, where I enjoyed the use of the University of London Table. I am grateful to Mr F. S. Russell, F.R.S., and the Plymouth Staff for much assistance, and in particular I have to thank Mr G. M. Spooner for the identification of many of the crustacea and Mr F. G. C. Ryder for help with the levelling of the area. Mr T. E. Hughes of Birkbeck College, London, was kind enough to identify the mites, and my special thanks are due to Prof. A. Graham for his continued kindness and encouragement while I was a research student at Birkbeck College during 1951 and 1952.

The Locality and Classification of the Habitats

The principal area studied was the rocky shore at Wembury, near Plymouth, where on either side of a small sand-beach two reefs run seaward in a south-westerly direction, Church Reef as discussed and surveyed by Colman (1932) and a similar reef immediately to the west. Both reefs are composed of Dartmouth Slates, and form near the landward edge a series of strongly projecting ledges, obliquely overthrust chiefly towards the north or north-west. The geological formation of slates is perhaps the most ideal for a study of crevice-dwelling animals, the successive slabs weathering apart to form narrow, oblique fissures often as much as 12 in. deep. The survey was confined to rocks above mean sea-level which is at 8·2 ft. above Chart Datum (C.D.) zero for Plymouth. Re-levelling of the portion of the area studied in detail was carried out, and the tidal levels have been taken to be those determined for Wembury by Colman (1932). As in that paper and in the work of Orton (1929), a height of 2 ft. has been allowed for the raising of effective water-level by the action of wave surge and splash. Following Colman (1932) heights above C.D. have been expressed throughout in feet.

A typical portion of the ground selected for sampling is shown in the profile diagram (Fig. 1) drawn from an outcrop of rock near the landward edge of the west reef. Here there is a vertical range up to 22 ft. above C.D. On the topmost outcrops of rock, above 20 ft. C.D., which were never covered by the tide or regularly subjected to wave splash, the surface is brightly daubed with the yellow terrestrial lichen *Xanthoria*. Down to *c.* 16 ft. C.D., a little way above MHWS the tarry black lichen, *Verrucaria maura*, encrusts most of the rock, being sprinkled at the lower limits with tufts of the bushy lichen *Pygmaea pumila*. From here down to *c.* 10 ft. C.D. there is a continuous zone of the barnacle *Chthamalus stellatus*, intermixed especially at the lower half with more tufts of *Pygmaea*. Below *c.* 10 ft. C.D. species of *Fucus* form a thick covering, in most places continuous, on the flatter rock surfaces, and there is

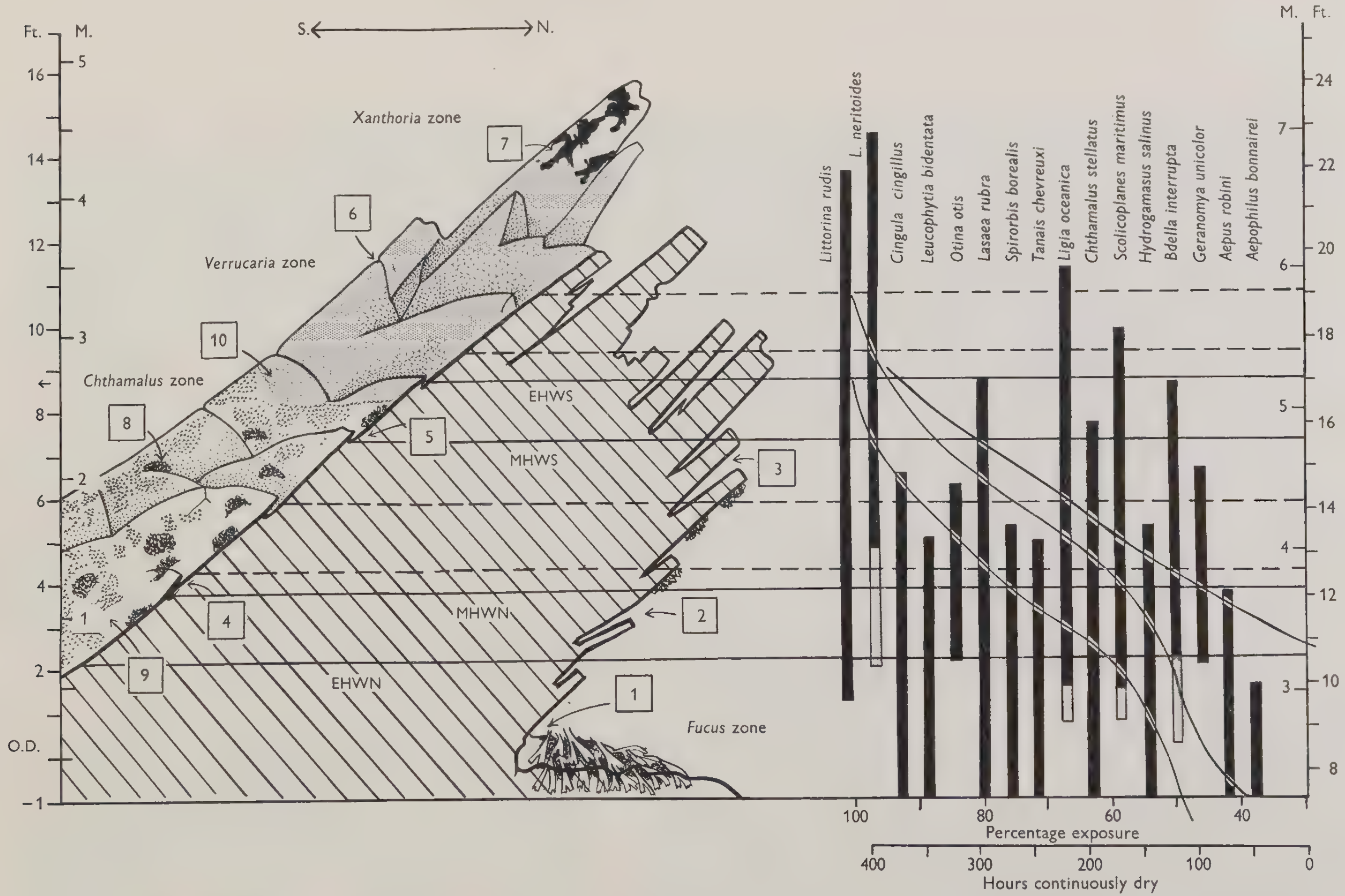

Ft.
M.
S.
N.
Xanthoria zone
Verrucaria zone
Chthamalus zone
Fucus zone
1
2
3
4
5
6
7
8
9
10
EHWS
MHWS
MHWN
EHWN
O.D.
Littorina rudis
L. neritoides
Cingula cingillus
Leucophytia bidentata
Otina otis
Lasaea rubra
Spirorbis borealis
Tanais chevreuxi
Ligia oceanica
Chthamalus stellatus
Scolicoplanes maritimus
Hydrogamasus salinus
Bdella interrupta
Geranomya unicolor
Aepus robini
Aepophilus bonnairei
Percentage exposure
100
80
60
40
Hours continuously dry
400
300
200
100
0

Fig. 1. Generalized diagram with a profile section of portion of an outcrop of Dartmouth Slates at the landward edge of the west reef at Wembury, showing typical positions for locations 1–10 referred to in Table I (p. 191). Tidal levels have been superimposed on the profile, the broken lines representing splash levels 2 ft. above the respective tidal heights. On the right are shown the vertical ranges of some of the commoner crevice-dwelling and other animals. A stippled portion of a column represents an extension of range on the exposed southward side. On the right are shown (below) the two curves for percentage of exposure at various levels, the 'splash curve' being the higher, and (above) the curve for the maximum period of continuous exposure (see Colman, 1932).

also in the damper and more shaded parts a closer turf of *Laurencia pinnatifida*. With the higher-occurring algae we have less concern in this paper; *Ascophyllum* enters the profile up to about 13 ft. C.D., with tufts of attached *Polysiphonia*, while farther up there is *Fucus spiralis* and finally *Pelvetia canaliculata*.

A leading factor coming into play in the arrangement of sites for crevice-dwelling animals is that of difference in aspect: the north overthrust of the slates gives on the sheltered northern side maximum protection from the evaporating power of the sun, and shaded conditions during ebb-tide. At the same time, on the north side, crevices running parallel to the direction of the thrust will tend to be deeper and more permanent than on the south side, where the weathered slates loosen in relatively small chips. These are frequently dislodged by extremes of temperature, stronger wave action, and in general more rapid weathering. The chief shelter provided on the south side is from the tufts of *Pygmaea pumila*. The effect of differences in aspect on the distribution of *Pygmaea* and *Chthamalus* and the fucoids on the north and south faces of the reef at Wembury has already been discussed by Evans (1947). The present results confirm Evans's statement that *Pygmaea* is thickest and most extensive on slopes facing in a southerly direction, but 'commonly well developed even on rough landward faces when these are covered with barnacles; it occurs also in caves and overhangs where these are exposed to strong water movement'. On the northern side *Chthamalus* is thinner in distribution, but continues on bare rock and within the lips of crevices to not far above its southern lower limit. On the southern side, below the *Chthamalus* zone, the most typical fucoid is *Fucus vesiculosus*, intermixed with some *Ascophyllum*. On the north side *Fucus serratus* reaches up to the lower limit of the barnacles without the intervention of a *vesiculosus* strip.

Taking into account differences in vertical height and differences in aspect and topography of the surface, and in the type of protective cover, on the rocks above 8·0 ft. C.D., it has been found possible to designate ten types of habitat, among which each of the points occupied by animals in this zone at Wembury can be fairly accurately assigned. Points **1** to **3** are taken on the shaded north side; **4** to **7** in crevices on the unshaded side, **8** in the tufts of the lichen *Pygmaea*, and **9** and **10** on the bare rock surface of the unshaded side. Table I and the reference numbers on the profile (Fig. 1) define these habitats sufficiently accurately for the classification to be applied over a fairly wide range of localities. It should be emphasized that each of the habitat numbers refers to a class of habitat, not to a single station sampled at a fixed location, and that the profile drawn in Fig. 1 has been generalized to include features of a number of such outcrops of rock on the western reef.

TABLE I. CLASSIFICATION OF HABITATS IN UPPER TIDAL ZONE AT WEMBURY

Type number	Description	Tidal levels (C.D.)
1	Crevices below upper limit of *Fucus vesiculosus* and *Laurencia pinnatifida*, shaded by damp algae	*c.* 10 ft. and below
2	Deep crevices on shaded (north) aspect in lower half of *Chthamalus* zone	EHWN to MHWN, *c.* 10·5–12·5 ft.
3	Deep crevices on shaded (north) aspect, near upper limit of *Chthamalus-Pygmaea* zone	Around splash zone of MHWN, *c.* 14·5 ft.
4	Shallow crevices on exposed (south) aspect, in lower half of *Chthamalus-Pygmaea* zone	EHWN to MHWN, *c.* 10·5–12·5 ft.
5	Shallow crevices on exposed (south) aspect, near upper limit of *Chthamalus-Pygmaea* zone	Around splash zone of MHWN, *c.* 14·5 ft.
6	Shallow crevices on exposed (south) aspect, in zone of black lichen, *Verrucaria maura*	Around splash line of MHWS, *c.* 17–19 ft.
7	Shallow crevices never reached by tide, exposed or sheltered aspect, in zone of yellow lichen, *Xanthoria parietina*	*c.* 20–22 ft.
8	In tufts of *Pygmaea pumila* on unshaded rock surface, in *Chthamalus-Pygmaea* zone (cf. Colman, 1940)	*c.* 11–14 ft.
9	Bare rock surface on unshaded (south) side, in lower half of *Chthamalus-Pygmaea* zone	EHWN to MHWN, *c.* 10·5–12·5 ft.
10	Bare rock surface on unshaded (south) side, in upper half of *Chthamalus-Pygmaea* zone	*c.* 13–16 ft.

METHOD—SAMPLING PROCEDURE

In attempting to give a quantitative estimate of the faunas present in each of the locations selected, several difficulties arise which are not experienced on the more uniform environment of a soft-bottomed shore, where the classical methods of notation are best brought into use. First, the environment is never entirely uniform or perfectly comparable in detail from one station to another. There is every gradation of such variable factors as differences of shade, differences of crevice substratum and differences in shelter from wave attack. On moving from one outcrop of rocks to another, often not even a rough replica of the previous environment is obtainable. Secondly, there is the extreme patchiness of many of the species themselves, even in environments that appeared to be approximately uniform. Thus the excursions made by the mite *Bdella* between crevices 4 and the bare rock surface vary extremely from hour to hour. *Anurida maritima*, to take another example, is sometimes present in several hundreds in one crevice and is likely to be quite wanting in another close by. With *Otina* and other animals aggregating together, perhaps a dozen may be found in a favoured part of a crevice and in a sample of another part altogether missed. Further, such very abundant dominants as *Lasaea rubra* vary within wide limits in apparently replicate areas—this is exemplified by Colman's three counts of *Lasaea* from 50 g. of *Pygmaea*. None the less, it would be misleading not to recognize in general a fairly constant relation—with large enough series of samples—between crevices of comparable locations. This tends to hold good over Wembury and other Plymouth localities as well, and to vary in roughly the same way with gradations in ecological conditions. There is one reservation: a single series of samples from any one chosen station may not give a complete picture, and variations within 30–40% are to be regarded as the normal range. On the profile drawn in Fig. 1, each location was sampled in at least three or four typical crevices, and the final distribution diagrams (Fig. 6) are also generalized from the results of two seasons' representative sampling over the whole area of the reef. It was thus found less useful to quote actual numbers of animals than to adopt the scale of densities as given in the figures. The area sampled in each case was a portion of the crevice of 15 cm.^{2}. On opening a crevice this was marked out in as quick time as possible, usually by laying down a sheet of stiff card and making marks at each corner. The upper and lower slate of the crevice were then sampled, giving a total area of rock surface of 2 × 15 cm.^{2}. The total population was sucked up into a 5 × 1½ in. collecting tube, with an entomology-bottle glass intake, and the contents examined in the laboratory. Swift-moving animals such as insects and crustaceans were secured first, and mites and insects drawn on to a layer of water to immobilize them from crawling out of the tube. Sessile organisms were then counted at leisure and tufts of *Pygmaea* were brought home and the animals collected in an enamel pan after remaining overnight in 1½% formalin.

The Habitats and Their Faunas

Location 2

Shaded aspect, deep crevices between EHWN and MHWN, lower limit
to middle of *Chthamalus* zone

A profile diagram with distribution figures for horizontal succession of animals in this type of crevice is given in Fig. 2. A description of location 2 will be given first, as this is the richest habitat with the most favourable combination of conditions for the development of the greatest number of species. It shows in a complete form a sequence represented elsewhere only partially or in fragments. Crevices here are seldom entirely dry between tides. With a northern aspect they receive maximum shade and the crevices slope down deeply, sometimes to a depth of 9–12 in. between the slates. They are rather sheltered from wave action, and the material of the substratum, both organic and derived from weathering, will tend to stay here longest and, as shown by Glynne-Williams & Hobart, there may be a rough grading of sediments on passing more deeply into the narrowest part of the crevice. Of ecological factors, the most significant is the absence of prolonged exposure (see Fig. 1) for more than a single intertidal period. The temperature within the crevice is both low and uniform throughout tidal exposure, and relative humidity remains always at—or just below—saturation point. The food supply comes apparently from two sources, suspended particles brought to the mouth of the

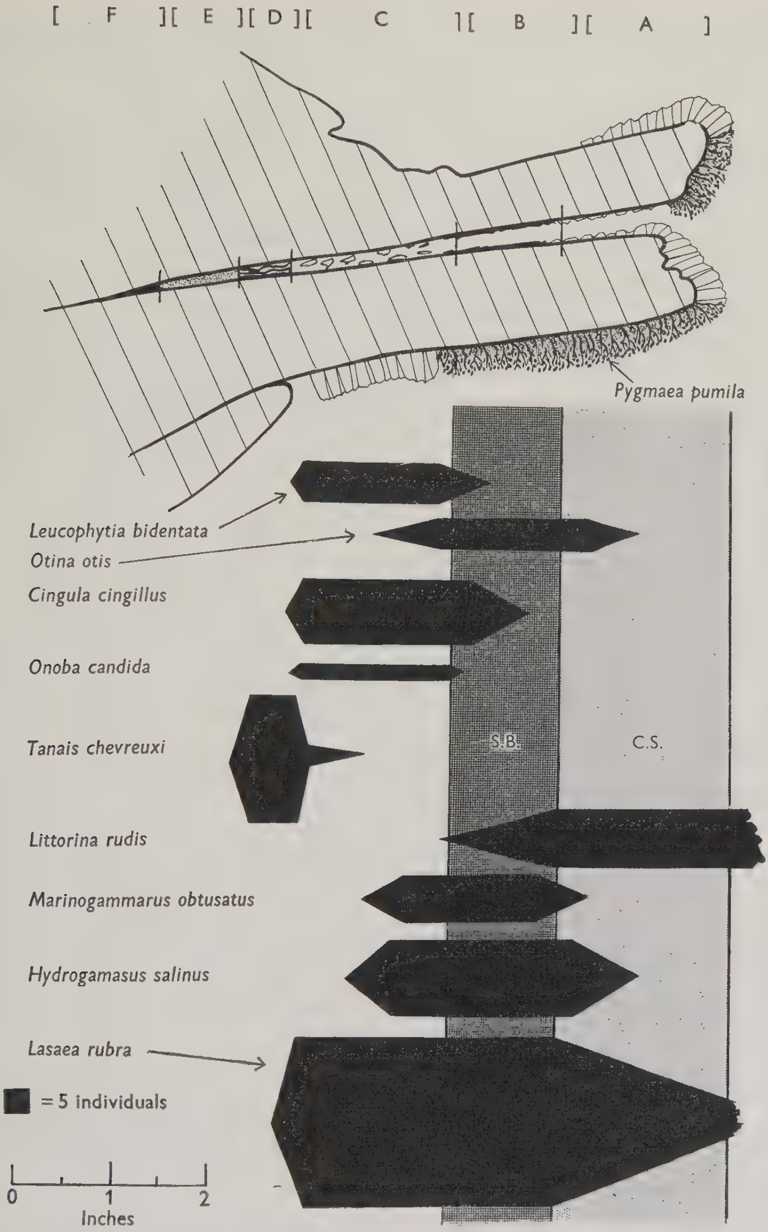

[F][E][D][C][B][A]
Pygmaea pumila
Leucophytia bidentata
Otina otis
Cingula cingillus
Onoba candida
Tanais chevreuxi
S.B.
C.S.
Littorina rudis
Marinogammarus obtusatus
Hydrogamasus salinus
Lasaea rubra
■ = 5 individuals
0
1
2
Inches

Fig. 2. Diagrammatic section of a typical intertidal crevice in Dartmouth Slate at location 2. The letters refer to the successive zones within the crevice discussed in the text. Distribution diagrams are given below for the occurrence of nine of the more abundant species of animal within the crevice. The blacked area represents the actual number of animals taken in typical samples, apportioned between the zones, the scale being given by the small black square which is equivalent to five individuals. The stippled areas indicate the approximate limits of the two encrusting organisms, *Chthamalus stellatus* (*C.S.*) and *Spirorbis borealis* (*S.B.*).

crevice by wave action and available throughout the crevice during tidal submersion; and organic detritus produced by sedimentation on the substratum of the crevice.

When prised open for examination, crevices of this type disclose a zonation of the fauna in successive horizontal strips, passing inwards from the opening. This is developed with greater or less completeness within each crevice as the depth allows. Not every crevice shows the complete series of zones recognized in Fig. 2. The primary factors which control zonation within the crevice appear to be the following: on passing deeper into the crevice penetration of light rapidly diminishes; wave splash with its combined effect of dislodging loosely attached animals near the mouth, and bringing a constant supply of micro-organisms within reach of filter feeders, rapidly ceases; the nature and composition of the substratum, both organic deposits and weathering products, change; and a lower and more constant temperature is attained with increase of depth. Relative humidity increases rapidly to saturation point at greater depth.

A zonation diagram shows ideally six zones, referred to as A–F, all of which but the innermost are inhabited. The outermost zone (A) is constituted by the projecting ledge of slate which is closely covered with *Chthamalus stellatus*. This barnacle extends from its thickest layer above the crevice lip round to within the opening, thinning out rapidly inside. Here the shells become rather paler. Smaller and more depressed, and more widely spaced, they are evidently limited within the crevice by the lack of direct wave splash on which the barnacle relies for feeding between tides. *Pygmaea pumila* occurs also in tufts and patches, usually most densely below the more shaded lip of the crevice. The whole zone grades outwards into the extended *Chthamalus-Pygmaea* zone which is the dominant outer covering at this level. With *Spirorbis* and *Ralfsia* in zone B these form the only encrusting organisms in the crevice. Freely moving larger animals which may shelter in the crevice at this zone include small specimens of *Patella vulgata*, large *Littorina rudis* and *Gibbula umbilicalis*. The two latter may go much deeper than zone A. They are not properly speaking crevice-dwelling animals at all and wander freely about on the rocks outside at this level.

At the second zone of the crevice (B) the barnacles have thinned out to final disappearance, but the penetration of light is evidently still strong enough to support an encrusting film of the brown alga *Ralfsia*, a fine greenish scum of *Enteromorpha* and often an encrusting pink *Melobesia*. Not much permanent deposit settles here and the crevice at this depth is kept rather clean by waves. The chief sessile animal is *Spirorbis borealis*, forming continuous sheets or patches, where the animals are still able to filter out nutriment from particles washed in by waves. This level in the crevice is the favourite site for *Otina otis*, which always, unlike *Leucophytia*, avoids regions of thickest accumulation of detritus. It prefers to browse upon a fairly clean surface, picking up organic fragments, and especially diatoms lodged by the wave-splash in slight irregularities of the surface. From the splash, *Spirorbis* filters out nutriment of much the same kind as *Otina* secures with its radula. *Otina* by no means confines itself to this zone. It is able to venture outwards, even while the tide is out, to the projecting ledge of barnacles. Here it crawls about actively, kept moist by splash, and often ventures among the barnacles on the under side of the lip of the crevice.

A deeper zone (C), beyond the *Spirorbis* fringe, occurs wherever a space of about ½ in. is still left between the roof and floor of the crevice. Small stones, pieces of shell, pebbles and coarser sand are able to lodge here. There collects also a deposit of fine clay-like weathering products, and a rich organic debris lodges in the interstices between the larger particles. The fauna is richer here. While *Spirorbis* is entirely lost, the chief species become *Cingula cingillus*, *Lasaea rubra*, *Leucophytia bidentata*, much reduced numbers of *Otina otis*, and many small individuals of *Littorina rudis*, which, however, is a good deal more common towards the mouth of the crevice. All the animals mentioned feed in various ways on the rich organic deposit. They include several species with purely marine affinities, such as *Lasaea rubra*, *Cingula cingillus* and *Littorina rudis*. A striking feature of the location, however, is the migration of a terrestrial element back between tide marks, into the specialized crevice locality. In smaller numbers, but usually in each crevice with one to a dozen specimens, are represented the myriapod, *Scolicoplanes maritimus*; the pseudoscorpion, *Neobisium maritimum*; a collembolan, *Anurida maritima*; a machilid, *Petrobius maritimus*; and the coleopteran, *Aëpus robini*; while the pulmonates *Leucophytia* and *Otina* are themselves supratidal rather than marine in affinities. In addition, the small, reddish brown marine mite, *Hydrogamasus salinus*, is present, often in immense numbers, forming one of the chief items of the species list. The air interlocked in small spaces at this level of the crevice is evidently sufficient to enable most of these animals to remain air-breathing between tides and to live compatibly side by side with marine members of the fauna. Some species, such as the mite and the collembolan, evidently retain air by a setose or hairy covering and are difficult to wet; but others, such as *Leucophytia*, are probably truly amphibious and breathe dissolved air from the sea water during high tides. Other members of the fauna in considerable numbers in zone (C) includes the amphipods *Hyale nilssoni*, *Marinogammarus obtusatus* (also found at the crevice mouth in the barnacle fringe), and frequently a specimen of the little teleost, *Blennius galeritus*, which regularly lodges in air-filled crevices between the tides. Among the isopods, *Sphaeroma serratum*, and *Naesa bidentata* are probably most important. Of the molluscs, another rissoid, *Onoba candida*, is regularly present, though in much smaller numbers than *Cingula*, as well as *Mytilus edulis*, pale brown in colour, up to 5 mm. in length and attached by byssus threads to the rock. The worms include several eunicids and terebellids, and also *Eulalia* *viridis*, which is typical both here and, perhaps even more, in the *Spirorbis* and *Chthamalus* zones. The high tidal nemertean, *Lineus longissimus*, is very frequent; there are often five or six in each crevice.

In a narrow, but fairly constant zone at a deeper level (D) the accumulation of sand and detritus becomes thick enough for the peracaridan *Tanais chevreuxi* to form its system of galleries. Each occupies the whole width of a narrow zone of compacted sand, about 1 in. across at most, and the burrows form a closely branching system in general parallel with the direction of the crevice. The chief animals accompanying *Tanais*, crawling back into deeper recesses between lodgements of muddy sand, are the isopod *Sphaeroma serratum*, reduced numbers of *Lasaea*, the shells now pale and transparent, and *Cingula* and *Leucophytia*.

Deeper still in the crevice, in zone (E), the clay deposit, chiefly the reddish product of the weathering of slate, becomes even more compact and finer in grade, and is penetrated by few animals with the exception of the deposit-feeding worms, *Amphitrite gracilis* and *Cirratulus cirratus* whose food-collecting tentacles extend outwards to the more open zones, C and D, in places where the *Tanais* zone is interrupted or unrepresented.

Zone E may extend backwards 3 or 4 in. into the crevice, after which, at the level F, the sheer faces of the slate laminae remain in contact or separated only by a film of red or yellow clay formed by weathering action. Here no space remains for penetration by living organisms.

Location 3

Shaded aspect, deep crevices at and around splash line of MHWN, near the upper limit of the *Chthamalus-Pygmaea* zone

The fauna here is a good deal less rich than that just described, and there are a number of differences in the impact of the environment. The chief ecological difference is evidently that of greater temperature fluctuation (see Fig. 3). There is a higher average temperature with longer exposure between tides and much greater insolation of rock surfaces. Consequently, relative humidity is lower because of both longer evaporation and lack of water-retaining deposits. The crevices tend to be less deep, and this is probably in some measure due to the different nature of the weathering: the slates tend to be sheared off or delaminated more by subaerial erosion than by the deeper action of water. Type 3 crevices may remain dry for a total period of up to 200 hr. within a tidal cycle. This ensures that deposit feeders will find conditions unsuitable. Many of the animals of the lower fauna have dropped out. Perhaps this has as much to do with unfavourable conditions of food supply as with temperature or humidity.

Leucophytia, which was typical of deposit feeders at 2, is lost entirely. *Otina* is still able to do well here and nestles among the barnacles or with *Lasaea*. It evidently reaches here the higher limit of its intertidal range. Like the limpet, *Otina* is able to crawl about actively and forage for wave-lodged diatoms or filamentous algae which are often found in its gut; it is able to feed by means of its radula from a rather clean-swept surface. Many tufts of *Pygmaea* still occur at the barnacle fringe at the mouth of the crevice. They shelter vast numbers of *Lasaea rubra*, which, as long as *Pygmaea* persists, is as abundant

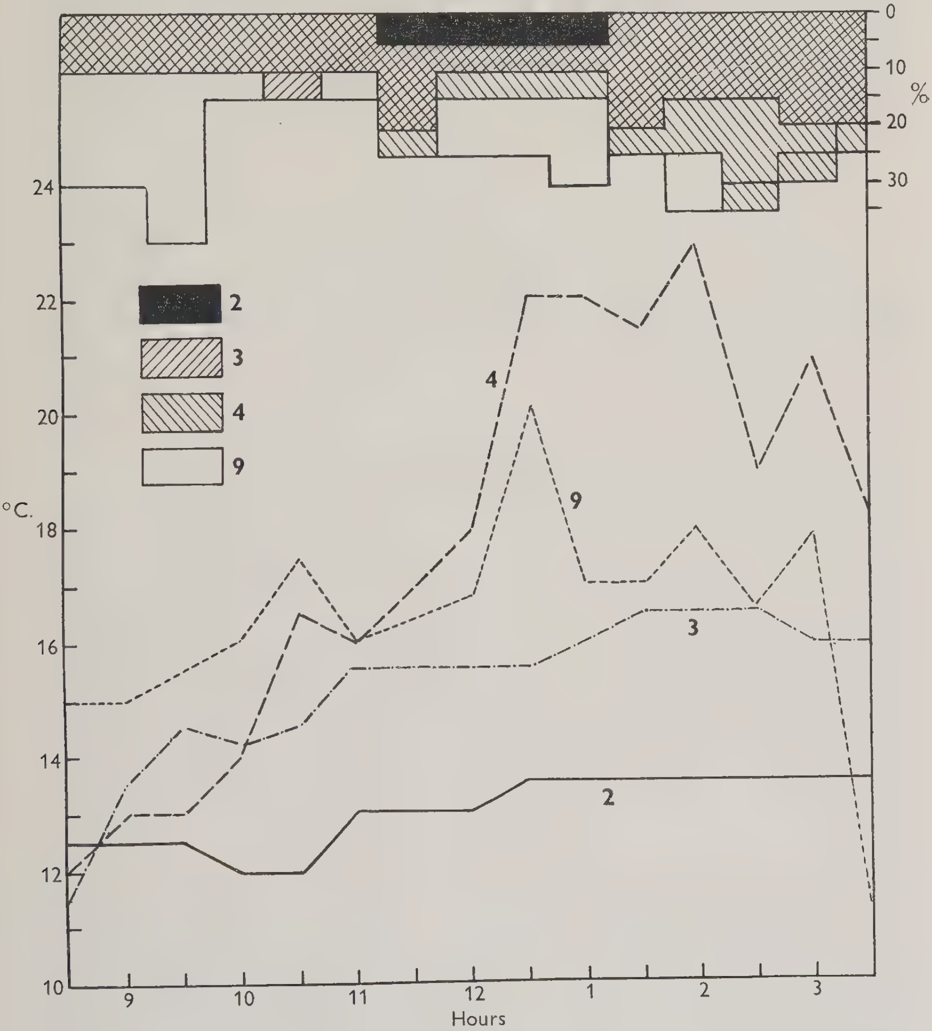


Fig. 3. Graphs showing half-hourly temperature readings over a complete intertidal period on a portion of the western reef at Wembury, on 18 June 1951; and (above) diagrams showing the percentage relative humidity by which the points selected fell short of saturation at the same intervals. Position (i), referred to in the text, was just above the bare rock surface at location **9**, and the remaining three stations are at typical positions of locations **2**, **3** and **4**.

as below. *Lasaea* is a suspension feeder and must at higher levels have a stringent tidal periodicity imposed on its feeding and other activities. *Littorina neritoides* is here almost as abundant as *Lasaea*. It prefers to nestle in barnacle shells and also presses in dense clusters as far into the crevice as it is possible for smaller individuals to go. There are still at this level many *Littorina rudis* and *Patella vulgata*, creeping into the crevice from outside.

Ligia oceanica now reaches its densest distribution, running about restlessly inside and outside the crevice. Its presence, together with the larger numbers of *Littorina neritoides* and the reduction of the deposit-feeding element, is the chief distinguishing feature from location 2.

Deeper in the crevice where the upper and lower slates are almost in contact there is little rich detritus and the population must wander abroad and capture what it can. The chief inhabitant, *Scolicoplanes maritimus*, is said to come out especially at night. The nemertine, *Lineus longissimus*, is evidently another such scavenger—its presence here, since it is apparently poorly protected against evaporation, is rather unexpected. *Ligia* itself is a scavenger and is stated (Glynne-Williams & Hobart, 1952) to be cannibalistic.

Location 4

Exposed aspect, shallow crevices just below MHWN, in lower half of the *Chthamalus-Pygmaea* zone

This location corresponds in height to 2. The chief differences are brought about by the topography of the slates forming the substratum; the crevices here are much less deep than on the north side, sheltered by small chips of slate only. With this is combined the greater wave action on the exposed seaward side, tending to scour the crevices clean of much of their debris and organic deposits. There is greater exposure to evaporation. The conditions found at 3 are here brought down on the unshaded side to a relatively much lower level and the resemblances of this location are chiefly with 3. A loosened slate is usually thickly clustered with *Chthamalus* at the edge of its under surface, while at a deeper level appears a crowded row of small *Littorina neritoides*, which occur also in or among the barnacle shells. There are also *L. rudis*, about half as numerous. *Ligia oceanica* is very active and quite numerous, reaching here its lowest tidal level. Farther in, as the crevice becomes narrower, appears a dense zone of *Lasaea rubra*, smaller and more deeply pigmented towards the outside, becoming larger and paler with depth. The substratum within the crevice is generally of reddish weathering products, with rather little organic material. *Tanais* is generally absent from these crevices which are scarcely deep enough, nor with sufficient supply of building material, to enable it to construct its burrows. Occasionally a small patch of mud may support a dozen or so *Tanais* and here conditions are often sedimented enough for *Cingula* which—with or without *Tanais*—may continue quite abundant. *Leucophytia* is present in these patches, but reduced both in size and number; on the unsheltered side this is about the upper limit of its extent. *Otina*, on the other hand, being less tied to thick deposits and doing better on cleaner ground, seems to tolerate these crevices quite well, often sheltering in empty barnacle shells. The local humidity at such points must closely approach saturation, although in general it may fall a good deal lower at 4 (Fig. 3). The carnivores or scavengers, *Machilis* and *Aëpus*, are represented here, but more poorly than at 2. *Anurida maritima* often swarms. Of the mites *Hydrogamasus* does not often turn up here, but the large reddish *Bdella* and *Cyrthydrolaelaps* are usually abundant, creeping out upon the heated rock surface over the barnacles.

Location 5

Exposed aspect, shallow crevices at splash line of MHWN, near upper limit of *Chthamalus-Pygmaea* zone, same level as 3

This zone is most readily comparable with 3, but with the unshaded conditions of south exposure, the environment is a good deal more harsh. In many ways the fauna approaches that of 6. The crevice is mainly filled with clean, pinkish brown weathering products along the joint plane of the slates, and little life exists in such places. At the outer part of the crevice, however, a fringe of *Pygmaea* helps to accumulate a small amount of organic deposits and provides shade for a narrow zone of *Lasaea rubra* and *Littorina neritoides* occurring within the mouth of the crevice. These are the two dominant animals. *Lasaea* is near the top of its range at this point and *Littorina neritoides* near its optimum. Both yielded over 200 individuals in the area sampled. *L. rudis* accompanies *L. neritoides* but is of small size and much less abundant. *Ligia oceanica* wanders about actively and specimens of the *Geranomya* larva were found which had probably strayed from the *Pygmaea* zone. *Scolicoplanes maritimus* was at times found here, but conditions seemed too dry for *Anurida maritima*. There are no *Tanais* and no worms. Deposit feeders are at a great disadvantage and the chief nutriment available consists of suspended particles borne by the waves.

Locations 6 *and* 7

6, exposed aspect, shallow crevices in the zone of black lichen, *Verrucaria maura*, above the barnacle zone, at 17–19 ft. C.D. 7, exposed or sheltered aspect, cracks in the rock never reached by the tide, in the zone of yellow lichen, *Xanthoria*, 19–21 ft. C.D.

These were the highest points sampled, and both were usually taken on the unshaded side of the profile. Conditions tend to be increasingly harsh and the fauna greatly impoverished. At 6, although there are no barnacles exposed on the surface, *Chthamalus* may exist in one or two sparse clusters in the crevices. *Pelvetia canaliculata* may still be growing at the surface, and its thallus and holdfasts provide shelter for a few animals (Colman, 1940). In the crevices *Littorina neritoides* is now by far the dominant organism, in practically terrestrial conditions reached only by the splash of high-water springs. *L. rudis* is still present but much more scarce. *Ligia oceanica* is active and plentiful, but none of the mites appear to persist. *Lasaea rubra* just reaches crevice **6** though it is outstripped in numbers by *Littorina neritoides*; its presence here is a remarkable achievement for a lamellibranch relying on suspension-feeding.

Finally, at **7**, small *L. neritoides* almost alone persists in an environment that is perfectly terrestrial. Occasionally small specimens of *L. rudis* make an appearance. There is little detritus or wave-lodged material; temperature extremes and lack of shade are very great; insolation is prolonged and high, and humidity very low. *Ligia* has practically disappeared and we find in its place small numbers of a slower terrestrial oniscid.

Location 8

In tufts of the lichen *Pygmaea pumila*, on the unshaded rock surface in the zone of *Chthamalus-Pygmaea*, at 11–14 ft. C.D. (cf. Colman, 1940).

Tufts of this lichen have remarkable water-retaining power and provide almost the only cover on the surface of bare rock for an extension of the faunas normally sheltering in crevices. The faunas of *Pygmaea* tufts were discussed by Colman (1940). The chief differences to be noted from crevice **4** are the smaller number of actual crevice-dwelling species to be found among *Pygmaea*, and the extraordinary abundance of two species, *Lasaea rubra* and the isopod *Campecopea hirsuta*. Colman remarks on the teeming masses of *Lasaea*, the highest number recorded by him being 12,140 in a sample of 100g. of damp lichen. A comparable order of density for 15 cm.^{2} of *Pygmaea*-covered surface was obtained during this survey, a typical figure being 898. *Campecopea hirsuta* is present here in numbers far greater than at **2** and **4** where it was also seen. Colman's figures of 2204 and 3772 per 100g. of lichen are of similar order to the sample of 494 for one square recorded in this survey. Of the acarines found here, not named by Colman, the red mite *Bdella* appears to be the most numerous. Of other migrants from the crevices, *Littorina neritoides*, *L. rudis*, *Anurida maritima* and *Petrobium maritimum* figure prominently. A new arrival is the tipulid dipteran *Geranomya unicolor*, of which large numbers of larvae were always found. This insect gave a remarkable demonstration of osmotic regulation in its resistance to a 5% solution of formalin in which it remained alive in immersed tufts of lichen overnight.

Locations 9 *and* 10

9, bare rock surface on unshaded side, between EHMN and MHWN near the lower level of the *Chthamalus-Pygmaea* zone. **10**, bare rock surface on unshaded side above MHWN near the upper limit of the *Chthamalus-Pygmaea* zone.

These locations are among the least favourable of all, and very few of the animals present in crevices at **4** and **5** are able to venture out here. The fauna is denuded of practically all species except tiny specimens of *Littorina neritoides* and *L. rudis*, which nestle in empty barnacle shells and are evidently able to crawl about at night and in conditions of greater shade. At **9** *L. rudis* is far predominant; at **10** it is reduced in numbers and *L. neritoides* has well overtaken it, being present in samples of more than 400 to a 15 cm.^{2} patch. *Lasaea rubra* has totally disappeared on the open surface. Evidently the evaporation rate is too great even with the tight closing of the shell during the withdrawal of the tide. Of the scavengers, *Ligia* probably comes out by night, as may also *Scolicoplanes*, but in the daytime *Bdella* alone remains swarming over the open rock surface in ceaseless activity.

Location 1

Crevices at upper limit of *Fucus serratus–Laurencia pinnatifida* zone, 10 ft. C.D., and below, shaded by damp thallose algae.

The fauna of crevices of this type presents a strong contrast with all of the locations higher up. The barnacles of the outer lip of the crevice are lacking, tending to be replaced by portions of the carpet of *Laurencia*, which creeps in slightly around the mouth of the crevice though never quite removed from the light. There follows inside the entrance to the crevice a rather narrow zone, which is often silted up, or—in cleaner conditions—supports a growth of encrusting red algae and bryozoa. *Ralfsia* never occurs here and there is usually too much silt for *Spirorbis*, which drops out almost entirely from the crevice fauna in most cases, but is still able to settle in abundance at this level on clean blades of *Fucus*. In this part of the crevice and farther in, on the organic deposits, *Lasaea rubra* still flourishes. Specimens are of large size and pale colour (Fig. 4). *Leucophytia bidentata* does well too, sometimes on very thick yellow-brown silt. *Cingula* is decidedly less common and *Otina* quite lacking. *Sphaeroma* is much less seen, the chief isopods being *Naesa bidentata* (♀=*Dynamene* spp.). There appear to be few *Tanais* in any crevices of this type. Entrants into the fauna which are never seen higher up are a small *Amphiura*, a pilumnid crab, *Anomia ephippium* of small size, and the very distinctive half-crab *Porcellana platycheles*. Tiny *Mytilus edulis* also appear, being sometimes also seen higher up at **2**. Most of the insects seem to be able to extend down to this zone where the crevice is open and clean enough to allow them, but the accumulation of moist silt is probably their limiting factor. *Aëpus* is on the whole less abundant than at **2**, but a new arrival is the heteropteran *Aëpophilus bonnairei* which is never present higher up and is distinctly a low-tidal form. With it occurs the bright green-bodied juvenile stage of the ectoparasitic isopod *Gnathia oxyuraea*.

The deeper part of the crevice is often permanently filled with waterlogged, but fairly stiff, sand and detritus. The most typical new entrant here is the burrowing lamellibranch *Hiatella arctica*, which forms horizontal galleries in the mud communicating by its red siphons with the outer part of the crevice. It is never found farther up, and with *Porcellana*, *Gnathia* and *Aëpophilus* forms a group of species diagnostic of this type of habitat. Among the worms present in the substratum *Perinereis marioni*, and *Eulalia viridis* are as common as above, accompanied by *Amphitrite gracilis* and *Cirratulus cirratus*, and—especially where there is much clay—*Phascolosoma minutum*. Groups of sabellariid tubes are common on the cleaner parts of the rock.

The Environment

The moisture content and the proportion of organic detritus present in the substratum of the crevice have been quantitatively assessed and discussed by Glynne-Williams & Hobart (1952). During the present work the two factors of relative humidity and temperature within selected crevices have been measured for a typical summer day. The temperature was recorded at each of four selected points on the outcrop of the west reef illustrated in Fig. 1, at half-hourly intervals during a complete period of intertidal exposure on 18 June 1951. This was selected as an average warm day on which conditions of temperature and evaporation approached their higher extreme. The meteorological conditions for Plymouth for the day included 'Sunshine 11·6 hr., Max. Temperature (in shade) 62°F, Sea Temperature 58·5°F, Barometer 30·08'.

The first series of temperature readings was taken at position (i), in air, 1 cm. from the exposed rock surface, on the unshaded south aspect corresponding to location 9. A second series of readings was recorded from within a crevice of type 4 on the unshaded side. On the shaded north side, observations of temperature were made at the bottom of a crevice of type 2, and higher up at the bottom of a crevice of type 3. Temperatures were recorded by Cambridge Instrument Company thermocouples, with a narrow flexible thermometer antenna which could be thrust into the full depth of a crevice, with care to avoid contact with the rock surface and without dislodgement of the slate or opening up of the surroundings. The results are presented in the lower half of Fig. 3. The internal temperature of crevice 2 remained low and uniform, rising by 0·5°C at 11.00 hr. to continue through the afternoon at its highest value of 13·5°C. In crevice 3 (where the intertidal exposure was longer) the temperature had risen at 09.30 hr. to 14·5°C, continuing, though less steadily, at an average of 2° higher than in crevice 2. On the exposed rock face at position (i), the temperature had risen to 15° within 30 min. after the tide had ebbed from that point, and, with a recession during the morning due to a light shower of rain, continued to rise throughout the morning to reach its peak of 20°C at 12.30 hr. In the crevice of type 4 the temperature, beginning at the same level as in crevices 2 and 3, rose rapidly during the early part of the morning till it exceeded at 11.00 hr. that of the exposed rock surface (i). This was apparently to be explained by the rapid heating up of the unshaded rock mass surrounding the crevice and the absence of the cooling effect of breezes and air currents that came into play throughout the day upon the exposed surface. The temperature in this crevice reached a peak of 24°C at 14.00 hr. and continued during the afternoon higher than, but with as great fluctuations as, that of (i).

Half-hourly measurements of relative humidity were made simultaneously with temperature readings at the same points by exposing small squares of cobalt thiocyanate paper, which was kindly supplied by Dr M. E. Solomon of the Pest Infestation Laboratory, D.S.I.R., Slough. Standardizing was carried out by exposure in desiccators in atmospheres of known relative humidity over solutions of potassium hydroxide of increasing specific gravity after the method of Buxton & Mellanby (1934). The colour standards so obtained were then compared with the reading of Edney paper hygrometers, and were regarded as having an accuracy of within 5%. Squares of humidity paper of $\frac{1}{2}$ cm. were introduced into crevices within the end of glass rods inserted into the crevices with care to avoid moisture condensed upon the rocky surface, and the papers, removed after 30 min. exposure, were placed in tubes of liquid paraffin for comparison in the laboratory.

The readings of relative humidity at (i) (location 9) and locations 2, 3 and 4 are presented on the upper portion of Fig. 3. Crevice 2 maintained much the highest relative humidity throughout the intertidal period, falling to 95% between the hours of 11.30 and 13.00, and remaining the rest of the time at saturation. The lower extreme is represented by the exposed rock surface at point (i) where the relative humidity fluctuated throughout the day, frequently falling to 65%. Intermediate values were obtained for crevices of type 3, which did not exceed 90% during the intertidal period and fell to 80% at 11.30 hr. and later during the afternoon. The type 4 crevice maintained a relative humidity similar to 3 during the early part of the day, later falling rapidly with rise in air temperature.

It would be an over-simplification to emphasize too strongly the apparently close correspondence between the peaks of temperature and the points of greatest saturation deficit. Obviously the variations of relative humidity form an ecological factor with very complex underlying causes. The water content of enclosed air may vary locally under the direct influence of the temperature of the atmosphere and of the substratum, together with variations in the amount of shade and exposure, and drainage of the crevice substratum (these two factors being dependent on the detailed topography of the ground); the percentage of intertidal exposure; the amount of wave-splash and the incidence of local breezes.

On the profile diagram (Fig. 1) have been superimposed curves for two sets of factors established by Colman (1932) with application to intertidal zonation at Wembury. The two lower curves are generalized compound curves showing the average percentage of exposure, for actual tidal level and for the splash level 2 ft. higher, during a complete tidal cycle. Such a curve is 'built up of

(1) the semi-diurnal tide curve and (2) the curves, having a wave-length of a fortnight, formed by the height of high and low waters as they vary between one set of springs and the next'. The upper curve represents 'the length of time during which any given level may be continuously dry during one fortnight (Vernal Equinox)'.

The last curve is applicable to an area lying above EHWNT, below which no place is continuously exposed during the whole of any tidal period. Referring to the upper limits of the ranges recorded in Fig. 1, it will be noticed that *Spirorbis borealis* reaches the splash zone of EHWNT; this species is one that appears to need daily, even at the lowest high neap tides, some amount of wave-splash and access to food. *Chthamalus stellatus* extends well above this level to MHWST. During neap tides it may remain out of the high-water splash zone at its upper limit, as may also *Lasaea rubra*. For the greater portion of a fortnightly tidal cycle, *Ligia oceanica* and *Littorina neritoides* appear to be quite independent of wetting by splash. For some of the animals whose vertical distributions are recorded in Fig. 1, we may extract Table II, expressing the average percentages of daily exposure at their upper and lower limits, and also the total number of hours during which these species—at the upper limit of their range—may remain continuously exposed during a fortnightly tidal cycle.

TABLE II. PERCENTAGES OF EXPOSURE, AND MAXIMUM NUMBER OF HOURS OF EXPOSURE AT UPPER LIMIT, FOR SOME CREVICE-DWELLING ANIMALS

	Average % of daily exposure (splash figures in brackets)		Maximum number of hours exposed (upper limit)
Species	Upper limit	Lower limit	
Ligia oceanica	100 (100)	57 (48)	Never submerged
Chthamalus stellatus	98 (90)	52 (45)	325
Tanais cavolinii	85 (66)	*	165
Hydrogamasus salinus	86 (70)	*	185
Spirorbis borealis	86 (70)	*	180
Lasaea rubra	100 (95)	*	370
Cingula cingillus	93 (83)	*	250
Littorina neritoides	100 (100)	82 (64)	Never submerged
L. rudis	100 (100)	60 (50)	Never submerged
Leucophytia bidentata	93 (65)	*	165
Otina otis	93 (78)	60 (49)	230

* Lower limit not observed in present survey.

DISTRIBUTION OF THE FAUNA

A species list for the habitats of the upper intertidal zone at Wembury is given in Table III. It has not been attempted to give actual numbers of single counts at particular crevices sampled, but rather to record conditions of density, from dominance to occasional presence. In the distribution diagrams (Fig. 6), quantitative data have been expressed by the scale of density shown on the figure. Each estimate is again not the result of any single sample, but gives a generalized picture from the results of two seasons' records from a large number of stations. Twenty-two species of animals are dealt with, the distributions being grouped according to taxonomic relationships, to give a comparative picture of the ecology of fairly closely related types of animals. In many cases, it will be realized that a uniform distribution of a species over the area of 10 cm.^{2} sampled is not implied. Thus, with *Lasaea*, individuals will frequently be crowded together in a narrow row a centimetre wide, and the remaining drier parts of the sampled square may show few or no specimens. An even distribution is much more a feature of crevices at 1 and 2. At 3, 4 and 5, the greater part of a crevice may be too dry to be inhabited, and this, rather than lower density at points of actual occurrence, accounts for the diminishing numbers of several of the species at these locations.

Some observations in more detail on the ecology of several groups of species of special importance in upper tidal crevices may be presented here. Glynne-Williams & Hobart (1952) have already recorded much useful data, especially about the terrestrial element in the fauna, in relation to feeding habits and position in the crevice.

Isopoda

The principal isopods are five in number, *Ligia oceanica*, an unnamed terrestrial oniscid of the wood-louse type, *Campecopea hirsuta*, *Sphaeroma serratum* and *Naesa bidentata*. Of these we may neglect the oniscid species which is of chief interest in showing the close approach to terrestrial conditions at 7. *Ligia* is typical of the upper half of the profile, most abundant at 3, next at 4, wandering about actively outside the crevice, especially at night. Its upper limit is probably governed by the dryness and shallowness of the crevice and the lack of adequate food supplies. It is thus almost absent at 7 and is in no sense truly terrestrial. It likewise appears to avoid *Pygmaea* (Colman, 1940) and is markedly reduced in numbers at 2 and quite absent at 1. Nicholls (1931 *a*, *b*) gives some account of its ecology and feeding, and this aspect has also been studied by Glynne-Williams & Hobart (1952). *Ligia* is replaced at 2, and also accompanied at 3, by the much more sluggish isopod *Sphaeroma serratum*. This species is far less tolerant of drying-out or temperature fluctuations. It is limited above at the MHWS line, and at its lower range just reaches 1. *Naesa bidentata* is found together with *Sphaeroma* in 2, but both these isopods are much more typical of deeply silted crevices and they extend downwards to reach their greatest abundance, in the area studied, at 1. They are found very little if at all on the unshaded south side. Numerically, by far the most important isopod is the small *Campecopea hirsuta*. As found by Colman (1940) in his sample from *Pygmaea*, this species is present in teeming numbers at 8, and there are always a few individuals represented at 2 and at 4 where they are likely to be overlooked on account of small size.

TABLE III. SPECIES LIST FROM INTERTIDAL CREVICES AND RELATED HABITATS

	Location type no.									
	1	2	3	4	5	6	7	8	9	10
Lineus longissimus (Gunnerus)	—	O	O	O	O	—	—	—	—	—
Small pink nemertine (indet.)	—	O	O	O	—	—	—	—	—	—
Perinereis marioni (Aud. and Edw.)	O	R	O	O	—	—	—	—	—	—
Unidentified eunicids	O	O	—	—	—	—	—	—	—	—
Eulalia viridis (O. F. Müller)	R	R	R	R	—	—	—	—	—	—
Harmothoë imbricata L.	R	—	—	—	—	—	—	—	—	—
Cirratulus cirratus (O. F. Müller)	R	O	—	O	—	—	—	—	—	—
Amphitrite gracilis Grube	R	O	—	O	—	—	—	—	—	—
Spirorbis borealis (Daudin)	O	D	—	O	—	—	—	—	—	—
Phascolosoma minutum Keferstein	O	—	—	—	—	—	—	—	—	—
Chthamalus stellatus (Poli)	—	D	D	D	O	—	—	—	—	—
Tanais chevreuxi Milne-Edwards	O	D	—	O	—	—	—	—	—	—
Sphaeroma serratum (Fabr.)	O	A	A	A	—	—	—	—	—	—
Naesa bidentata (Adams)	A	R	O	O	—	—	—	—	—	—
Campecopea hirsuta (Mont.)	—	R	—	O	—	—	—	D	—	—
Ligia oceanica L.	—	O	A	A	A	R	R	O	O	O
Unidentified terrestrial oniscid	—	—	—	—	—	—	O	—	—	—
Gnathia oxyuraea (Lillj.)	R	—	—	—	—	—	—	—	—	—
Hyale nilssoni (Rathke)	*O	—	—	—	—	—	—	—	—	—
Marinogammarus obtusatus (Dahl)	—	A	A	O	O	—	—	—	—	—
Porcellana platycheles (Pennant)	R	—	—	—	—	—	—	—	—	—
Juvenile pilumnid crab	R	—	—	—	—	—	—	—	—	—
Anurida maritima (Guérin)	O	A	A	A	O	O	—	O	—	—
Petrobius maritimus Leach	O	R	R	R	—	—	—	—	—	—
Aëpophilus bonnairei (Sig.)	R	—	—	—	—	—	—	—	—	—
Micralymma marinum (Stroem.)	—	O	—	O	—	—	—	—	—	—
Aëpus robini (Laboulb.)	R	A	—	O	—	—	—	—	—	—
Unidentified caddis	—	—	—	—	—	—	—	O	—	—
Geranomya unicolor	—	—	—	O	—	—	—	D	—	—
Scolicoplanes maritimus (Leach)	—	R	R	R	R	O	—	—	—	—
Neobisium maritimum	—	O	—	—	—	—	—	—	—	—
Cyrthydrolaelaps hirsutus Berlesé.	O	O	—	—	—	—	—	—	—	—
Hydrogamasus salinus Laboulbene	A	D	O	O	—	—	—	—	—	—
Bdella interrupta Evans	—	O	O	A	O	—	—	—	A	—
Protereupetes sp.	—	O	—	A	—	—	—	—	A	—
Anomia ephippium L.	O	—	—	—	—	—	—	—	—	—
Mytilus edulis L. (juv.)	R	O	—	—	—	—	—	—	—	—
Lasaea rubra (Montagu)	D	D	D	D	D	O	—	D	—	—
Hiatella arctica (L.)	D	—	—	—	—	—	—	—	—	—
Tapes pullastra	O	—	—	—	—	—	—	—	—	—
Acanthochitona crinitus (Pennant)	O	—	—	—	—	—	—	—	—	—
Littorina neritoides (L.)	—	—	D	A	D	D	A	R	R	D
L. rudis (Maton)	—	A	A	A	A	A	O	R	D	A
L. littoralis (L.)	O	O	—	—	—	—	—	—	—	—
Gibbula umbilicalis (da Costa)	—	R	R	—	—	—	—	—	—	—
Cingula cingillus (Montagu)	A	D	D	R	—	—	—	—	—	—
C. cingillus var. *rupestris*	*D	D	O	O	—	—	—	—	—	—
C. semistriata (Montagu)	R	O	—	—	—	—	—	—	—	—
Onoba candida (Brown)	R	O	—	—	—	—	—	—	—	—
Onchidella celtica (Cuvier)	*O	R	—	—	—	—	—	—	—	—
Otina otis (Turton)	—	A	R	R	—	—	—	—	—	—
Leucophytia bidentata (Montagu)	A	A	—	O	—	—	—	—	—	—
Blennius galeritus L.	O	O	—	—	—	—	—	—	—	—

D, dominant; A, abundant; R, regularly present; O, occasionally present; —, absent.
* Occurs at Whitsands but not seen at Wembury.
Nomenclature as in Plymouth Marine Fauna (Marine Biological Association, 1931).

Tanais chevreuxi

In choice of site, both with respect to tidal height and to depth in the crevice, this is one of the most restricted animals encountered in the present study. Its requirements appear to be a sandy mud, richly silted by weathering products and organic detritus, with an admixture of sufficient coarse sand to enable its galleries to be given a compact structure. As described above (p. 196) it presses deeply into crevices to a point where there is room for one layer only of animals between the two slates. Its appearance is an indicator of fairly mature conditions of succession in locations of type 2. The only other place where it is at all abundant is sometimes at 4. It never appears at 3, where its absence is one of the criteria of this type of crevice, and it apparently becomes cut out at 1 where the conditions of silting up and waterlogging of the crevice are too extreme. Dennell (1937) has described the feeding habits and ecology of *Apseudes talpa*, and this is the species of tanaidacean found by Glynne-Williams & Hobart at Anglesey. It would be interesting to know something more about the distribution and ecological requirements of *Tanais chevreuxi*, and to compare its occurrence at Wembury with that of the allied species, *T. cavolinii*, which would seem not to occur in crevices.

Mites

These may be taken together as a group, though they are by no means without clear-cut ecological distinctions, as between the species mentioned at Wembury. On the shaded side, in greatest concentration at 2 and extending abundantly down to 1, the chief mite is *Hydrogamasus salinus*, small in size and polished tan-brown. It is accompanied by a species of *Protereupetes*. On the south exposed side, most at home at 4 and 5 and wandering on to bare rock at 9, though found also on the shaded side, are two larger and more spectacular mites, the bright red *Bdella interrupta* and *Cyrthydrolaelaps hirsutus*. These are able to extend well up the shore into terrestrial conditions. They are air-breathing, evidently able to retain atmospheric air imprisoned between their fine setae. They appear to be widely foraging scavengers and also some of the chief carnivores of this area of the reef. The present writer has no direct observations on their feeding, but reference should be made to Glynne-Williams & Hobart (1952), and to previous observations quoted by them.

Lasaea rubra

This little lamellibranch is the most numerous animal in the upper shore. Its astonishing abundance at location 8, in tufts of *Pygmaea*, has already been remarked upon by Colman. It is no less dominant at 1, 2, 3 and 4, where it crowds closely into the tighter parts of the crevices almost as thickly as it is possible to aggregate. As found by Colman, numerical differences between successive samples of such large numbers may be very great, and on the distribution diagrams (Fig. 6) its fluctuations do not show up, as wherever it occurs it may be expected to exceed 200–300 in the 2×15 cm.^{2} area sampled. It seldom if ever occurs uniformly over the whole of such a patch, except at 2 and 1, where larger-sized individuals may be more evenly spaced out. At 3 and 4 its most typical occurrence is in a mass of smaller individuals pressed close together in a narrow line at the shallowest and innermost portion of the crevice. Figures for three of a number of samples taken were, for example, at a crevice of type 2, 865, at 8, 898, at 5, 455, and most samples at 1 and 4 totalled well over 400. The distribution of *Lasaea* is terminated abruptly by its complete disappearance above the level of 6; and unlike the Littorinas it is more or less coincident in its upper level with the barnacle zone, but even here it needs more shelter—which is evidenced by the presence of not a single specimen on the open surface at 9 and 10. It does best where it can take shelter by pressing into the smallest cracks or into empty *Chthamalus* shells, but it goes down well below the *Chthamalus* level, as may be seen from the present results at 1, and from Glynne-Williams & Hobart. Colman (1940), in his account of the fauna of the holdfasts of the larger brown algae (*Ascophyllum*, the fucoids and *Laminaria digitata*), finds no abundance of *Lasaea*, as in *Pygmaea* tufts. Though this bivalve is plentiful in crevices of type 1, below the *Chthamalus* zone, its maximum numbers always appear to occur in the neighbourhood of *Chthamalus* and *Pygmaea*.

There is a rather marked differentiation between *Lasaea* from two different groups of habitats. In the well sheltered conditions at 1 and 2 and the deeper parts of crevices at 3, the average size is much larger, length of shell up to 3 mm. or more and the colour pale. At 4, 5 and 6, and especially in tufts of *Pygmaea* at 8, the mean length is smaller. There is a larger element of tiny individuals below 1 mm. in length and the predominant group is of $1\frac{1}{2}$–2 mm. (Fig. 4). The shells are all much darker brown or reddish purple in colour.

Lasaea rubra anchors itself in a confined space by a tiny byssus, and it appears to show the same thigmotactic behaviour as *Otina*. It is probably claustrophilic for the same reason as *Otina*, both tending to remain aggregated in chinks and small cavities with the greatest local humidity. It appears to be entirely a suspension feeder; even in the richest organic deposits at 2 and at 1, it appears to have no particular modifications for sucking-up detritus in the manner of a deposit feeder. At 6 and at 5 it may undergo a total dry period for at times as long as 300 hr. continuously. Here the amount of nutritive deposits must be exceedingly small, and *Lasaea* must have become highly efficient at capturing the suspended particles splashed up to it by wave action in its relatively short period of contact with the water. Popham (1940) has given a detailed account of the pallial cavity of this bivalve. In addition, many questions still present themselves with respect to the physiology and cycles of activity in *Lasaea*. At its upper limits this lamellibranch must show

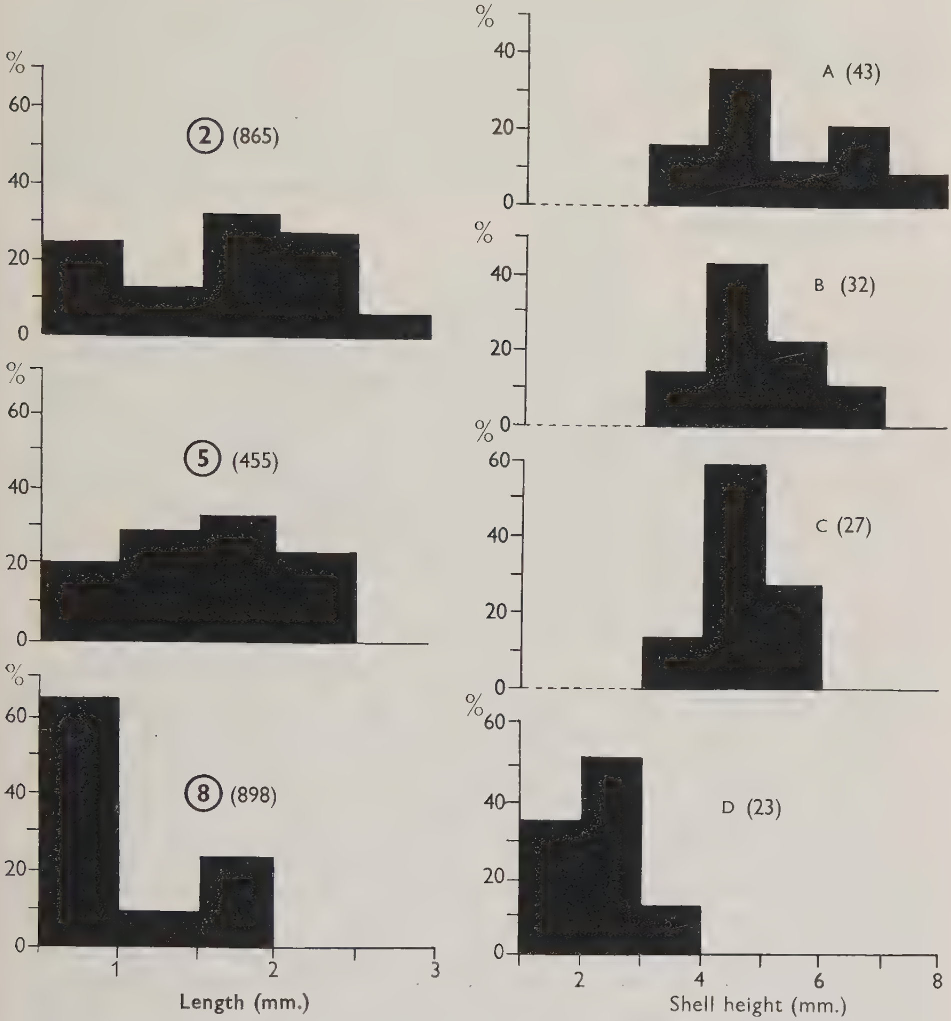


Fig. 4. Left. Histograms showing the percentage distribution of five size-groups of *Lasaea rubra*, in typical samples from locations of types 2, 5 and 8 (actual numbers in samples are bracketed). Right. Histograms showing the size composition of four samples of *Leucophytia bidentata* (actual numbers are bracketed). A, B and C are from deep shaded crevices of type 2 at Wembury. Individuals below 3 mm in height were not measured for Wembury; repeated observations at 1 and 2 indicated that they form only a minor fraction of the population. Sample D is from shallow crevices in limestone conglomerate on the wave-exposed Hoe front. The level sampled was at the lower limit of the *Chthamalus* zone.

a tidal periodicity very strongly impressed on the whole of its feeding and pallial activity. It would be of great interest to find how this double diurnal rhythm has affected the action of the gut, the presence of the crystalline style, and the functional rhythm of digestive diverticula. Further work might be carried out on the action of the ciliary systems of the mantle cavity during long periods of exposure and on the possible effect of fluctuations in pH of the contents of the mantle cavity. Variations in behaviour and physiology might then be examined from a comparison with *Lasaea* living in conditions of longer submersion at 1 and 2.

Leucophytia bidentata

This species is a primitive pulmonate belonging to a family centred in supratidal salt marshes and in brackish estuaries. I shall give elsewhere an account of its functional morphology. Within the family Ellobiidae, it belongs to a group of species that has returned to life between tide marks; the shell and the animal are entirely white, and the species is strongly light-avoiding. It is a deposit feeder on organic detritus, but it is without doubt somewhat selective; it does not take sand particles into the gut in appreciable volume, though its food is a good deal more mixed than that of the related *Otina*, which always prefers the cleaner fringes of crevices. Among the gastropods at 3 it comes next in order of abundance after *Cingula cingillus*, which it almost always accompanies, though in much smaller numbers, generally one-third to one-half as many. *Leucophytia bidentata* reaches its greatest numbers in well sedimented, humid crevices at 2, and individuals attain their greatest length (more than 5 mm.), here and at 1. At 3 and at 4 it is present in much reduced numbers and smaller size (*c.* 2–2·5 mm.) (Fig. 4, right). It spreads also, with *Cingula*, to the under sides of clean stones in upper tidal rock pools, as on the stone beach at the Hoe front near the Laboratory. It was taken also at Noss Mayo in very brackish high-tidal pools with *Enteromorpha* present as an indicator of much fresh water seepage. Here it was of tiny size.

In the crevices of the area studied the upper limit of *Leucophytia* is evidently governed both by unfavourable conditions of humidity and reduction of the supply of detritus. In lower crevices, as at 1, it seems to flourish and to attain a large size on the thick mantle of yellowish-brown detritus which seldom dries out at low tide. Glynne-Williams & Hobart also reported it as a low-tidal crevice animal at Anglesey. It is undoubtedly very much better able to withstand submersion than *Otina*, and evidently employs the supra-anal lobe of its pallial skirt in respiration. The 'pulmonary' pallial cavity was never found to be filled with water. Unlike *Otina*, *Leucophytia* has no preference for wave splash or well oxygenated water; it crawls feebly, and has no power to resist the dislodging action of water movements. Specimens survived for several weeks in the still water of a tidal tank with no other aeration than a twice-daily exposure to the atmosphere.

Otina otis

This interesting little mollusc is also a representative of the most primitive of the marine pulmonates. Its distribution on British coasts appears to be limited to the west and south-west, and Glynne-Williams & Hobart do not record it from Anglesey. Its British and west European distribution is to be discussed elsewhere. At Wembury it is one of the most discriminating animals in its choice of environment. It is almost entirely confined to crevices and their immediate neighbourhood, within a rather short tidal range. At its maximum occurrence at 2 it avoids the more heavily silted conditions in the deeper parts of the crevice, and creeps out upon the *Spirorbis-Ralfsia* zone, though it often intermingles with *Leucophytia* farther in. A favourite habit is to creep farther out on to the barnacle fringe in shaded spots, and to nestle in empty *Chthamalus* shells like *Littorina neritoides* (Fig. 5). It is above all a lover of clean conditions, and is never found in situations where excess detritus is present. Of all the molluscs, save the littorines, it has the highest lower limit, and it does not accompany *Leucophytia* down to the silted conditions at 1. It is best developed at 2, but unlike *Leucophytia* it can live favourably at 3 and it is present in greater numbers than *Leucophytia* at 4. Its mode of life is not unlike that of a limpet; with its strong radula it scrapes up wave-washed diatoms and algal filaments growing at the mouth of the crevice. Farther down in the crevice it is able to secure richer detritus, but its stomach contents are always finely graded, with a high proportion of diatoms, and seldom include particles of coarse detritus.

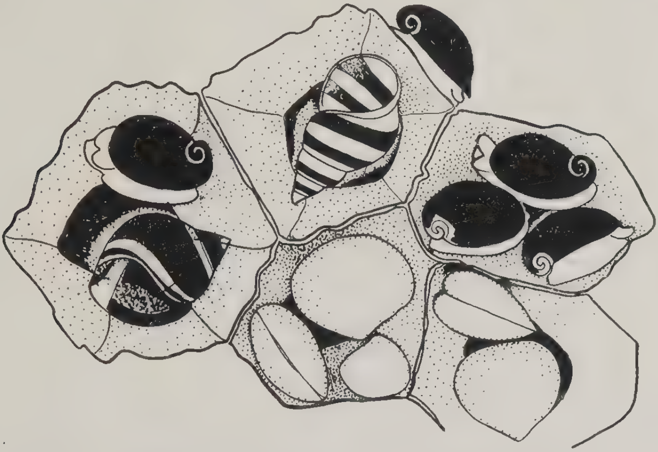


Fig. 5. A group of dead *Chthamalus stellatus* shells removed from within the lip of a crevice of type 4, and viewed from underneath to show the occurrence within them of *Otina otis* and *Littorina neritoides* (black), *Cingula cingillus* (banded) and *Lasaea rubra* (white).

Both *Otina* and *Leucophytia* are usually present in crevices at 4 where they can evidently tolerate several hours of lowered humidity. Sometimes also at

3 the saturation deficiency may approach that of 4. Perhaps both species are rather better able to withstand a shorter period of greater saturation deficiency at 4 than a longer one of lesser deficiency at 3. However, a period of humidity as low as 80% at 4 may represent a condition to which *Otina* and *Leucophytia* are not exposed for significantly long periods. Both take the fullest advantage of moist cracks where the humidity may remain locally high, and the humidity conditions of a single crevice may show wide local variations, with sheltered regions of high humidity which the thermocouple used did not reach.

The behaviour of *Onchidella celtica*, another primitive pulmonate which occurs in crevices of type 2 at Whitsands alongside *Otina*, may be compared with the above results. Fretter (1943) observed that the animals 'lived for several days in a relative humidity of 95% before showing signs of desiccation. They were quite active with the pulmonary aperture frequently open.' In 90% relative humidity they showed no apparent effect of dryness for 3 days. In 80% all after 2 days appeared shrivelled and inert. In *Onchidella celtica* tolerance of low humidity for limited periods is evidently great: in mid-August the animal was seen to leave the shady rock and travel over sunny surfaces. Their ratio of exposed surface to volume is of course much less than in the smaller *Otina*, and evaporation is probably retarded by the thick leathery integument which, aided by secretory glands, compensates for loss of shell. Animals of *Otina* and *Leucophytia* kept for 12 hr. in a desiccator at 90% relative humidity appeared shrivelled and dried out. Fretter states that *Onchidella* is 'completely akinetic, making no attempt to aggregate in regions of optimum humidity when placed in a humidity gradient of 75–95%.' *Otina*, on the other hand, reacts quickly to the equally unfavourable extremes of either a fall in the humidity of the atmosphere or of complete immersion. Jeffreys has observed (1869) that 'the animal is a restless little creature and when put into sea water crawls directly out of it.' The same active response is made when a crevice is opened and a group of *Otina* are subjected to the evaporating power of warm air. They crawl quickly into the shelter of a deeper part of the crevice, or, if exposed for more than 2–3 min., again become inactive, cease to crawl and clamp the shell tightly against the substratum.

The upper limit of *Otina* at *c.* 15 ft. C.D. is probably governed not by reduction of food supply but by prolonged saturation deficiency. Its lower limit at EHWN is fixed by its intolerance of long submersion and its dislike of silt. Still water or submersion for more than 3 or 4 hr. seem adverse. *Otina* does not survive in the still waters of a tidal tank, preferring the broken and well oxygenated water received from wave-splash. Both pigmentation of the shell and the streamlined limpet shape indicate that *Otina* is well at home on the clean-swept outer fringe of the crevice, and is well adapted to resist both wave attack and light. Its habit of thigmotaxis, with a tendency for a group of animals to cluster together in contact, or to nestle in a barnacle shell, is probably in part an adaptation for reducing water loss by evaporation. In addition, the habit of negative geotaxy, clinging by preference to the upper slate of a crevice, may assist it to take advantage of the atmospheric air longer retained in this part of the crevice.

DISCUSSION

Ecological factors

Any discussion of the causes affecting the distribution of crevice-dwelling animals in relation to their environment must necessarily be tentative and inconclusive. We know little at present of the mechanisms by which shore animals respond to changes in their surroundings, and distributional data are at present available only for the few localities studied in detail by the writer, and previously by Glynne-Williams & Hobart. In the absence, however, of any attempt, before these two papers, to assess the importance of factors operating in crevice habitats, the writer felt justified in advancing the ideas induced by the present study. Their preliminary nature will be evident; and their greatest use may be to form a basis of more exact future work.

Of the ecological factors with a direct effect on the fauna of crevices, the principal ones are probably relative humidity and the presence of adequate food supply, whether in the form of organic deposits or of wave-borne micro-organisms. Other factors may act to a large extent indirectly by their influence on one or other of the first two, though perhaps the effects of temperature and the dislodging power of wave action may also be direct. The most important factors influencing relative humidity are probably percentage of daily exposure, maximum longest period of exposure during a tidal cycle, maximum air temperature, incidence of breezes and amount of shade and rate of drainage of the crevice. The last two will, of course, vary complexly with the whole topography of the surrounding rock mass. With the contrasts at Wembury between southerly and northerly aspects, with crevices overthrust towards the north, both sets of factors—shelter and the structure of the rock mass—may be combined in one or other of their two sets of extremes. For the majority of the fauna both factors are in their most favourable combination on the north, and most rigorous towards the south. It would be possible in rocks overthrust towards the south, with deep crevices thus facing south, to examine the effects of separating rather than combining these two factors. Relative humidity, temperature and duration of exposure are easily measured (see above), and on the moisture content and organic detritus in crevices, quantitative data have already been given by Glynne-Williams & Hobart.

In the presence of a saturated atmosphere in crevices, the distribution of animals is likely to be governed chiefly by food supply. Here the controlling factors are probably the direct action of wave swell and splash, bringing suspended micro-organisms within the range of suspension feeders, and shifting detritus from the crevices. The influence of the surrounding rock structure is here very variable with situation, and very complex.

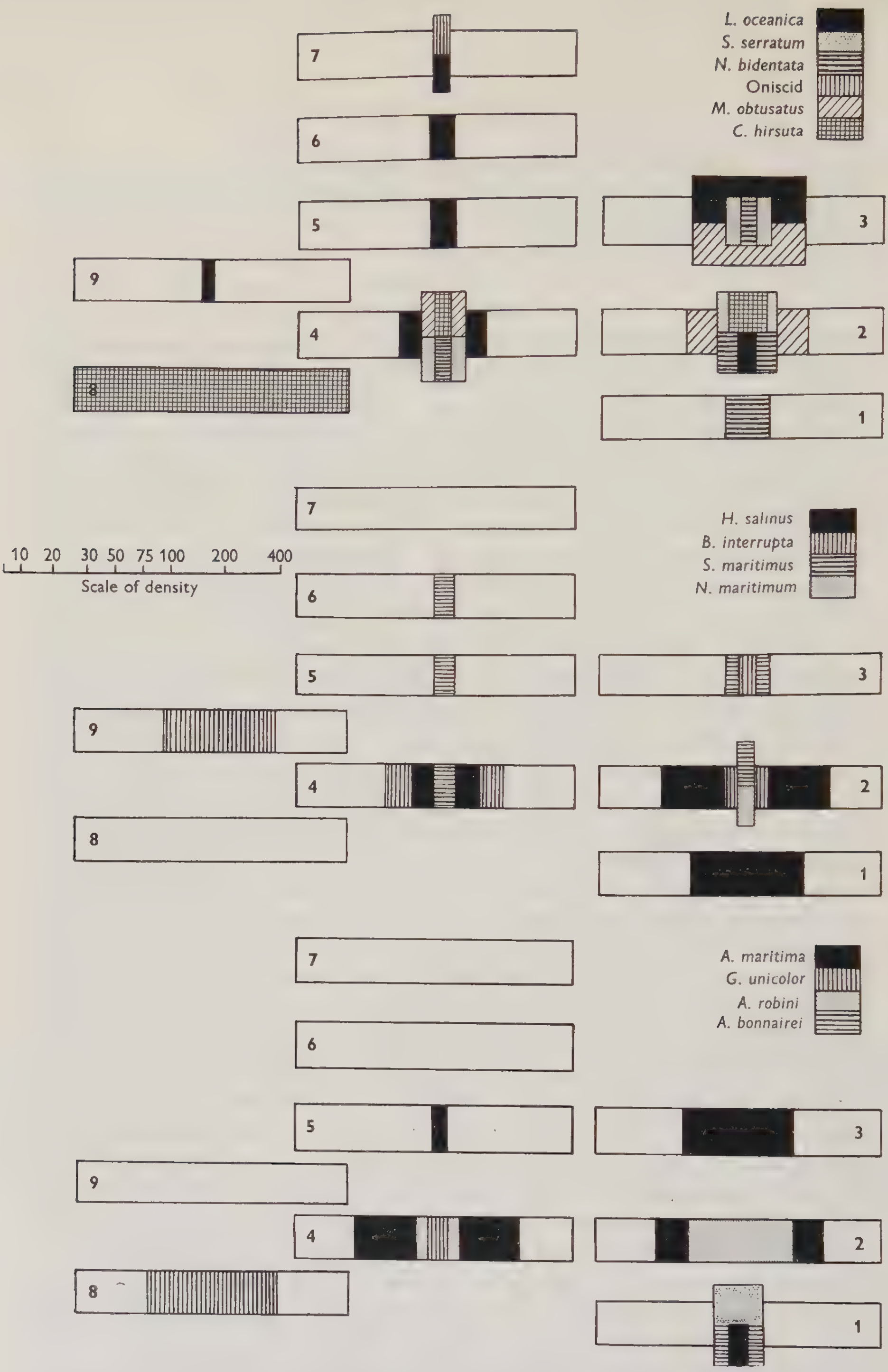
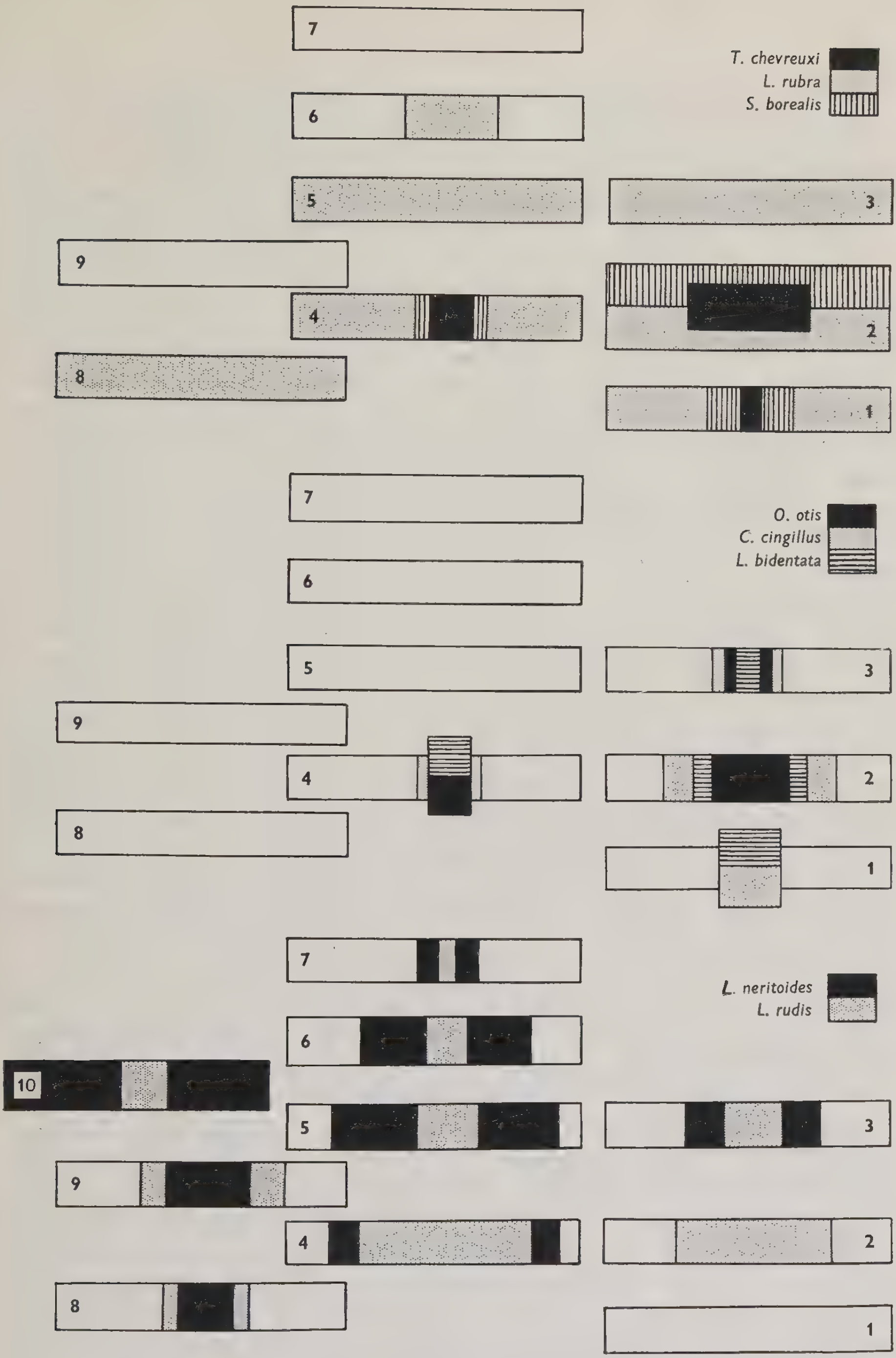

L. oceanica
S. serratum
N. bidentata
Oniscid
M. obtusatus
C. hirsuta
7
6
5
9
4
8
3
2
1
H. salinus
B. interrupta
S. maritimus
N. maritimum
10 20 30 50 75 100 200 400
Scale of density
7
6
5
9
4
8
3
2
1
A. maritima
G. unicolor
A. robini
A. bonnairei
7
6
5
9
4
8
3
2
1

Fig. 6. Diagrams showing the quantitative distribution of twenty-two species of animals in crevices and related habitats at Wembury. The species are the following: *Ligia oceanica*, *Sphaeroma serratum*, *Naesa bidentata*, terrestrial oniscid, *Marinogammarus obtusatus*, *Campecopea hirsuta*; *Hydrogamasus salinus*, *Bdella interrupta*, *Scolicoplanes maritimus*, *Neobisium maritimum*; *Anurida maritima*, *Geranomya unicolor*, *Aëpus robini*, *Aëpophilus bonnairei*; *Tanais chevreuxi*, *Lasaea rubra*, *Spirorbis borealis*; *Otina otis*, *Cingula cingillus*, *Leucophytia bidentata*; *Littorina neritoides*, *Littorina rudis*. They have been arranged in



six groups, mostly according to approximate taxonomic relationships. Each horizontal panel represents a numbered location according to the classification adopted in the text. They are arranged in approximate sequence according to conditions of shade and height for easy reference to Fig. 1. Location 10 is included only in the case of the littorinids. The scale of densities adopted is shown inset. The area shaded for each species is to be regarded as extending across the middle of the panel, and the smaller areas as superimposed on, and not interrupting, the larger.

Of other factors which may act directly, the dislodging power of surf is likely to have little direct effect on animals within the crevice, and those venturing out on to the ledge are well adapted for clinging to the surface, as well as being like *Otina* protected by pigment from the light. In some cases, such as with *Onchidella* and *Otina*, intolerance of sediment and preference for a clean substratum no doubt determine both the lower limit of the species and the horizontal position taken up within the crevice.

The boundary at Wembury between the zone of *Chthamalus stellatus* and *Fucus vesiculosus* or *F. serratus* appears to mark a true ecological threshold with respect to sheltered faunas living in crevices. Ecological factors of different kinds come into play, and exert the dominant roles, above and below this line. Above this mark, zonation within the crevices appears to be determined by the direct factors (i) relative humidity, and (ii) richness and adequacy of the food supply. Of the animals typical of this region, above *c.* 10 ft. C.D., stopping just below EHWN, we find the two components of the fauna recognized by Stelfox (1916), representatives of predominantly terrestrial groups, and representatives of groups that are chiefly marine in their affinities. Of the 'terrestrial' animals here that do not also extend farther downward, there is a rather short list, *Ligia oceanica*, *Bdella interrupta*, *Scolicoplanes maritimus*, *Anurida maritima*, *Geranomya unicolor* and *Otina otis*. As we shall see, this by no means exhausts the full terrestrial list, which has an almost equal number of species extending downwards into the *Fucus* zone. Of the animals belonging to the marine component of the fauna, there is a small group equally typical of the level above EHWN. Of these, *Littorina rudis*, *L. neritoides* and *Chthamalus stellatus* seem to go very little lower. Others, like the rest of the terrestrial list, do go lower, though *Lasaea rubra* often tends to thin out in its distribution below 2. *Cingula cingillus* continues only in reduced numbers, and *Spirorbis*, and *Tanais* when present at all, show altered modes of occurrence.

Below the upper limit of *Fucus vesiculosus*, the great ecological difference, from the point of view of the fauna at present being discussed, is the presence of dense thallose algae in a continuous mat, and the consequent shelter of the crevices from drying out and from the full force of wave action and scouring. There will thus be fewer differences as between north and south sides, resulting from aspect and the direction of thrust of the substratum. The humidity of the crevice is always saturated, organic detritus is always abundant, and the problem of excess sedimentation takes the place of that of a deficiency of food supply. There will further be much less stratification within the crevice, of the kind which was brought about at higher levels by different amounts of drying out and wave action on detritus, with increasing depths within the crevice. In general, those animals which are able at a higher level to tolerate the heavily sedimented parts of the crevice are able to extend below the *Fucus* upper level, at least in reduced numbers. There is thus an overlap into this zone of a group of animals extending downwards below the *Fucus vesiculosus* upper limit, but reaching their most typical development and greatest numbers above that line. Among these may be placed *Leucophytia bidentata*, *Cingula cingillus*, *Lasaea rubra* and in small numbers *Tanais chevreuxi*. Conversely, on both sides of the line but dwindling on extent into crevices in the barnacle zone is a group of animals containing *Amphitrite gracilis*, *Cirratulus cirratus*, *Perinereis marioni* and probably most of the worms, with the exception of *Spirorbis borealis*, *Lineus longissimus* and *Eulalia viridis* which, in crevices, are more at home in the barnacle zone.

For the terrestrial element that does not extend below the lower barnacle limit, namely *Otina otis*, *Bdella* sp., *Scolicoplanes maritimus* and *Neobisium maritimum*, (though the latter is very sparse in numbers and it is difficult to be exact about its distributional limits) the limiting factor is probably increased sedimentation of the crevices which is especially important in restricting *Otina*. For land-derived crevice animals as a whole there does not seem to be a general lower barrier at this zone. *Aëpus robini* and *Aëpophilus bonnairei* exist happily at Wembury at the level of 1, and Glynne-Williams & Hobart have shown their extent to or beyond MLWS at Anglesey. They no doubt retain aerial respiration as do the mite *Hydrogamasus salinus* and the thysanuran *Anurida maritima*; reliance on aerial conditions is probably possible at much lower levels and with much shorter exposures between tides than at EHWN. This group of insects, and of the mites—at least *Hydrogamasus salinus*, are thoroughly intertidal. Their extent into this zone is by no means dependent on a precarious compromise with marine conditions, and they are content with a short intertidal exposure for the renewal of air supplies. The arachnid *Neobisium*, the chilopod *Scolicoplanes*, the mite *Bdella*, and probably also the tipulid *Geranomya* are less well adapted for conditions in the lower intertidal zone. But, on the whole, the uppermost zone—locations 3, 4, 5 and 6—is characterized in largest numbers not by members of the terrestrial element but by marine animals (*Lasaea rubra*, *Littorina rudis*, *L. neritoides* and *Ligia oceanica*) which have evolved a tolerance of longer exposure.

Of the upper limits of range of the species recorded in the present survey, the most significant is at the *Fucus vesiculosus*–*Chthamalus stellatus* boundary. A large and varied fauna of type 1 location—not in detail listed here—cuts out between crevices 1 and 2. Crevices 2 never contain, for example, ophiuroids, pilumnid or porcellanid crabs, *Anomia* or *Hiatella*. The most interesting disappearance here is that of the bug *Aëpophilus bonnairei* which is not a high-tidal form at all. An upper limit for a further group of species lies between crevices of types 2 and 3. The animals disappearing here are *Spirorbis borealis*, *Cirratulus cirratus*, *Amphitrite gracilis*, *Tanais chevreuxi*, *Hydrogamasus salinus*, *Aëpus robini* and *Neobisium maritimum*. *Leucophytia* is here greatly thinned out, and we find almost the last of *Otina* and *Cingula*.

Above the level of 3 and for the most part in 4, the fauna, with the exception of *Scolicoplanes*, is not on the whole terrestrial in composition, but consists of an enterprising filter-feeding and suspension-feeding marine element like *Chthamalus* and *Lasaea*, or like the Littorinas, which are equipped with a strong scraping radula. Here they are almost entirely free of predator pressure, if this is ever indeed a significant factor, which is perhaps to be doubted in this area, with the exception of *Nucella lapillus* in relation to *Chthamalus* at its lower boundary. Animals of the terrestrial element are less adept than those above listed at obtaining their food suspended in sea water. For the most part, both for the sake of an adequate food supply and for protection from desiccation, they must go farther down in the intertidal zone and enter more fully into tidal conditions. Here they have almost all become carnivores or scavengers. The reasons for the falling out of the terrestrial element, at the highest levels surveyed, are probably connected with both food supply and humidity, disadvantages which hard-shelled, filter-feeding or radula-browsing animals have overcome.

Ecological Succession

Crevices between slates are in constant course of opening up by the loosening and ultimate detaching of a block of slate, usually the underlying one, by the action of water and weather. We have an accurate idea of the way in which a crevice may widen during the process of weathering, and though it was not possible in two seasons to detect the succession of the fauna at a single location, we can, by observing the zones present in crevices of different depths, build up a picture of the changes that must take place in the composition of a crevice fauna. Intertidal crevices provide a good example from the sea shore of an ecological succession in the words of Allee's definition (1949), 'the progressive sequence of replacement of communities over a given point, area or locality'. A crevice is first colonized as a small chink on the rock surface affording shelter to animals, and ultimately becomes deeply thrust into the rock mass before it is eliminated by the breaking away of the slate. The changes thus brought about in the substratum by weathering may be referred to as a 'physiographic succession'; the entire series of stages from bare rock through the deepening crevice with a final return to bare rock, form a 'sere'. The changes in the composition and relationships of the fauna may be regarded as constituting together the 'ontogeny' of the community. At its fullest development a crevice in Dartmouth Slate will reveal the five or six rather well defined zones illustrated in Fig. 2. As stages in succession we may recognize several phases—of *colonization* with the opening up and penetrating of a new crevice by successive groups of animals, new zones becoming represented with greater depth; of *maturity* as represented with complete horizontal zonation as in Fig. 2; and of *regression* by the gradual opening up and drying out of the crevice, leading to the final dropping of the slate and the re-occupation of the bare rock surface by *Chthamalus* and *Pygmaea*. It is difficult, without long-term observations, to estimate the rate at which succession may proceed. It is probable, however, that with the relatively rapid weathering of a foliaceous rocky substratum, the sequence of stages in Dartmouth Slate may move faster than is usual on rocky shores. The human investigator with his hammer and chisel becomes at times a biotic factor that appreciably speeds up the course of succession.

The diagram (Fig. 7) attempts to sum up the course of events at the level of a type 2 crevice, where the mature fauna is able to develop its fullest zonation. For comparison with the stages in a complete sere, we may refer to the other types of location, where the final stages represent stages intermediate in the succession at 2. Here the ecological conditions are seldom favourable to give expression to all the stages of a complete sere. Thus, each of the types 3, 4, 5, 6, 7 and 8 broadly correspond to stages passed through in the development of a crevice at location 2. At stage (i) in the suggested succession, a small chink is established in the slate, and barnacles at once extend into it, together with *Littorina neritoides* and *L. rudis*. *Pygmaea* also takes advantage of the shaded surface overhanging the crevice above, though it normally reaches only just to the typical level of 2. A comparison of this stage may be made with locations of types 7 and 8. Wherever *Pygmaea* grows a little detritus lodges deeper to it, so that at (ii) *Lasaea rubra* is able to nestle in a protected niche, with still a few *Littorina neritoides* as before, even in the slightest chink, but generally with a predominance of *L. rudis*. *Scolicoplanes maritimus* can lodge itself deeply in the extending crack, and *Ligia* shelters here and runs about freely on the exposed surface. Comparison may be made between this stage and the final condition at locations 5 and 6.

At (iii) the crevice has pushed back a little farther, more organic material has accumulated together with an admixture of fine sand, and *Tanais* becomes established in its galleries to form the zone lying deepest, with, immediately outside it, *Lasaea*, now accompanied by *Cingula* and a few *Leucophytia* (cf. location type 4). At (iv) the weathering has extended deeper. The *Tanais* zone tends to be pushed back as always to the narrowest, most recently opened parts, though there is sometimes, as in the complete succession, a zone of muddy sand or inorganic weathering products inhabited by deposit-feeding worms whose tentacles extend through gaps in the *Tanais* zone. Farther outwards in the crevice there is now room for members of the zone (C) of Fig. 2, with *Leucophytia*, *Cingula*, *Sphaeroma*, *Naesa* and most numerous of all, *Lasaea rubra*. Still farther out becomes established the innermost lighted zone (B), which is open enough not to be clogged with detritus. Here there is an encrusting mantle of *Spirorbis* and *Ralfsia*, and *Otina* is best developed here as well as extending into zone (C).

At maturity the crevice is established with its full zonation as shown at Fig. 2. Regression of zonation takes place upon further opening up and drying out of the crevice, by the action of greater wave-scouring, and penetra-

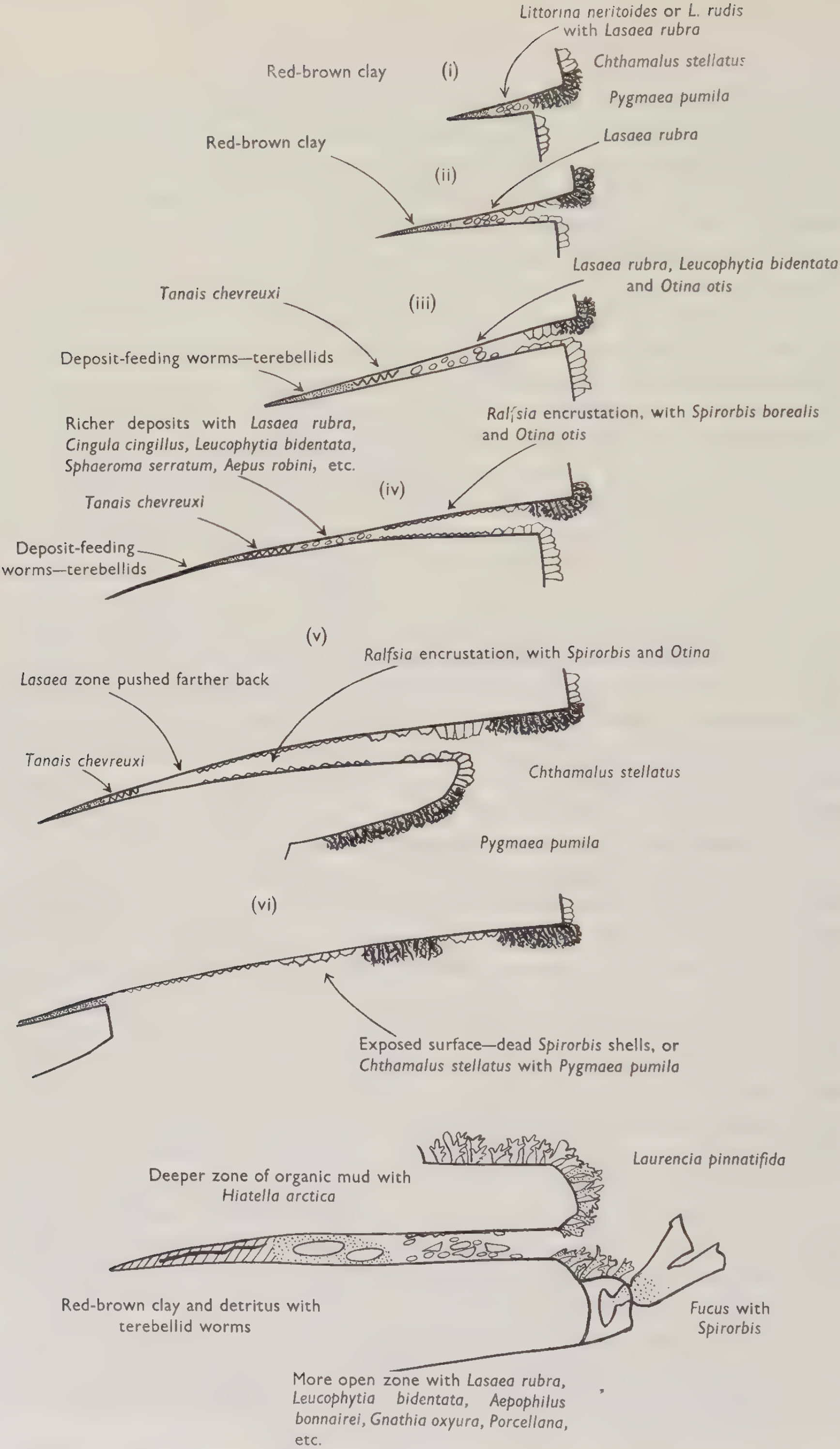

Littorina neritoides or L. rudis with Lasaea rubra
Red-brown clay
(i)
Chthamalus stellatus
Pygmaea pumila
Red-brown clay
Lasaea rubra
(ii)
Lasaea rubra, Leucophytia bidentata and Otina otis
Tanais chevreuxi
(iii)
Deposit-feeding worms—terebellids
Richer deposits with Lasaea rubra, Cingula cingillus, Leucophytia bidentata, Sphaeroma serratum, Aepus robini, etc.
Ralfsia encrustation, with Spirorbis borealis and Otina otis
(iv)
Tanais chevreuxi
Deposit-feeding worms—terebellids
(v)
Ralfsia encrustation, with Spirorbis and Otina
Lasaea zone pushed farther back
Tanais chevreuxi
Chthamalus stellatus
Pygmaea pumila
(vi)
Exposed surface—dead Spirorbis shells, or Chthamalus stellatus with Pygmaea pumila
Laurencia pinnatifida
Deeper zone of organic mud with Hiatella arctica
Red-brown clay and detritus with terebellid worms
Fucus with Spirorbis
More open zone with Lasaea rubra, Leucophytia bidentata, Aepophilus bonnairei, Gnathia oxyura, Porcellana, etc.

Fig. 7. Scheme to illustrate the course of ecological succession, (i) to (vi), as shown by crevice faunas at a type 2 location at Wembury. The lowest figure shows for comparison a crevice in the fucoid zone (location 1).

tion of the heat of the sun. The level of zones (B) and (C) is all the time pushed farther back, while at the bottom of the crevice where the slates are almost in contact is a fragmentary or complete zone of *Tanais* (D). Sometimes extending behind the *Tanais*, at times intermixed with (C), is the zone (E) inhabited by deposit-feeding terebellids.

With still greater loosening of the slate (vi) the crevice may become too open to wave-scouring to retain rich deposits, and during exposure to air the humidity of its atmosphere may fall. In such a crevice *Ligia*, *Littorina rudis* and sometimes *Gibbula umbilicalis* of the outer zone are able to push in more deeply, and the zone (C) of *Lasaea* moves towards the bottom of the crevice, with *Leucophytia*, *Otina* and *Tanais* tending to be lost. As a final stage of weathering, the whole lower slate may break away, and the under surface of the topmost slate may then sometimes remain encrusted with dry *Spirorbis* shells, or—at a higher level—acquire barnacles and tufts of *Pygmaea*.

Locations of type 1, as has been pointed out, fit less well into such a system as is outlined above. These crevices have a longer submergence, and are more permanent, with a more stable and less obvious zonation within. The chief ecological feature is the much greater silting-up and the permanent water-logging of the deposit. The communities consist of a narrow zone of *Laurencia*, spreading into the mouth in place of *Pygmaea*, an open silted zone with porcellanid crabs, ophiuroids, etc. (see p. 201), and finally a zone of compacted sand and detritus where *Hiatella* and deposit-feeding worms are typical.

Comparison with other localities

Glynne-Williams & Hobart (1952) discussed the faunas of crevices in green mica schists. Both here and at another locality examined in the Plymouth area, the Dartmouth Slate reef at Whitsand Bay, the mode of foliaceous weathering and the succession and composition of the fauna seem to be essentially the same as at Wembury. Two unexplained local differences at Whitsand Bay are the presence there, alone in the Plymouth area, of *Onchidella celtica*, and the replacement of *Cingula cingillus* by its white variety *rupestris*. At the two Dartmouth Slate localities, the crevices are probably on an average deeper than at Anglesey, which may account for the greater range of horizontal zonation. Five inhabited zones could be recognized, of which the first two probably best correspond to Glynne-Williams & Hobart's 'outer zone', the remainder to their more heavily silted 'inner zone'. Crevice faunas have also been examined at Rum Bay and Jennycliff Bay (Staddon Grits), Drake's Island (igneous intrusion), and Kingsand (felsite). Here, the large blocks of rock develop narrow vertical joint planes, and though the fauna is similar to fragments of the Wembury zonation, the full succession seems to be seldom developed.

The chief feature of the fauna lists for the Plymouth area and for North Wales is the detailed similarity in composition and in the dominant species represented. Thus for the intertidal fauna, *Lasaea rubra*, *Littorina rudis*, *Cingula cingillus*, *Leucophytia bidentata*, *Ligia oceanica*, *Campecopea hirsuta*, *Sphaeroma serratum*, *Anurida* (*bisetosa* in North Wales, *maritima* here), *Cyrthydrolaelaps hirsutus*, *Bdella interrupta*, *Hydrogamasus salinus*, *Neobisium maritimum*, *Scolicoplanes maritimus* and *Eulalia viridis* are all characteristic of both regions. *Aëpus robini*, *Aëpophilus bonnairei* and *Gnathia oxyuraea* are equally characteristic at a lower level. This list may probably be a familiar one on most British shores of similar formation, and, as remarked by Glynne-Williams & Hobart, the greater number of its species are those not commonly found outside crevices. We find, as it were, a 'crypto-zonation' in crevices, equally characteristic with the zonation on exposed surfaces. With the damping down of differences in wave action, light and evaporation, zonation in crevices may be more uniform over considerable vertical heights than the surface zonation. Nevertheless, with differences in aspect and in depth of crevices, many gradations are brought about, and—in particular, the influence of aspect and topography—makes the concept of 'critical levels' of less value, with its demarcation of zones more strictly in terms of tidal level.

One or two important absences of Plymouth animals from the Anglesey list of crevice fauna may be noted. *Spirorbis* is not stated to play any part in crevice communities, and there is no mention at all of *Littorina neritoides* at higher levels. Its lack of mention is probably due to the smaller emphasis placed by Glynne-Williams & Hobart on the distinctive features of crevices in the upper part of the zonation. *Otina otis* and *Tanais chevreuxi* are evidently entirely absent at Anglesey. *Tanais* seems roughly to be replaced ecologically by *Apseudes talpa*, which was not found at all in crevices at Plymouth.

Otina otis has a rather restricted geographical distribution, being a north-west European and British species only. Jeffreys (1869) gives Normandy, Brest, Quiberon, Piriac and Loire Inférieure for its French distribution, and in England the Devon and Cornish coasts. It extends to south Wales, south and north Eire and Northern Ireland. Moore (1937) records it on the Isle of Man. *Otina otis* is evidently a southern species and its range would seem in Britain to follow fairly closely that of *Chthamalus stellatus* with which it lives in fairly intimate association. It is apparently absent, however, from the north Scottish coast, accompanying *Chthamalus* only as far as the Firth of Clyde. At Carmel Head and North Stack, Anglesey, *Chthamalus* was found by Southward (1951) at its minimal distribution for the Irish Sea, and no *Otina* at all were noticed by Glynne-Williams & Hobart. The range of *Otina* in the English Channel a little exceeds that of *Chthamalus*, and a record by Jeffreys of a dead shell from Northumberland seems to require reinvestigation.

It is possible to suggest a tentative scheme of crevice zonation, based on the Plymouth results, which may with some modifications, as at Anglesey, be found to hold good over a large part of British shores where the nature of the rock mass is suitable to it. The following regions can be defined in terms of approximate tidal levels, though these are subject to wide variation, and the sequence of zones is always most complete on the shaded overhanging side, and modified as has been shown on the unshaded side.

(i) Above EHWS, where *Littorina neritoides* is dominant and often alone.

(ii) From MHWS to MHWN, where *L. rudis* enters into the zonation with *neritoides* and *Lasaea rubra* become abundant and dominant. *Ligia oceanica* is also highly typical, and associated with *Chthamalus stellatus* appears the marine pulmonate *Otina otis.*

(iii) MHWN to the bottom of the *Chthamalus* zone, where the Littorinas drop out, and a numerous deposit-feeding element appears. *Lasaea rubra* is completely dominant; deposit feeders like *Leucophytia bidentata*, *Cingula cingillus* and *Otina otis* are most abundant here, and *Spirorbis*, *Eulalia viridis*, *Aëpus robini* and *Hydrogamasus* are typical.

(iv) A special habitat at the same level, the tufts of *Pygmaea pumila*, still dominated by *Lasaea rubra* which is accompanied by *Geranomya unicolor* and *Campecopea hirsuta* in vast numbers.

(v) A zone of changed conditions below the *Fucus vesiculosus* upper limit, where *Otina* and *Tanais* disappear and *Cingula cingillus* thins out. There is a continued dominance of *Lasaea rubra*, abundance of *Leucophytia*, *Aëpus* and *Hydrogamasus*, and a new appearance of *Aëpophilus*, *Porcellana*, *Hiatella*, *Gnathia* and many other low-tidal forms.

SUMMARY

This paper presents the results of a study of the animals dwelling in crevices and related habitats on the upper portion of an intertidal reef of Dartmouth Slates at Wembury. The oblique overthrust of the slates towards the north provides a series of habitats on the north and south aspects differing in respect of exposure to the sun and to wave action, and in the depth of the crevice and the composition of its substrate. A series of ten types of microhabitat has been recognized, and an account given of the quantitative distribution and mode of occurrence of the typical species of animals present at each type of location. The nature is discussed of the horizontal zonation within a typical crevice under the most favourable conditions on the shaded side. Fuller ecological notes are provided for several of the ecologically more important species, especially the molluscs *Lasaea rubra*, *Leucophytia bidentata* and *Otina otis.* Of the environmental factors, temperature and relative humidity during the time of exposure have been measured at intervals throughout a single intertidal period. A tentative discussion is given of some of the ecological factors which may be of limiting importance in these habitats, and a scheme is presented of the probable course of ecological succession with the weathering of the rocky substratum and the progressive deepening of the crevice.

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J. Mar. biol. Ass. U.K. (1954) 33, 225–255
Printed in Great Britain

THE NERVOUS CONTROL OF LUMINESCENT RESPONSES IN POLYNOID WORMS

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(Text-figs. 1–10)

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Introduction

Some preliminary observations on luminescence in polynoid worms have been presented in a previous paper (Nicol, 1953). These animals produce light in scales (elytra) which cover the dorsal surface of the body. The source of the light lies in a layer of unicellular epithelium on the lower surface of the scale. Histologically, this tissue consists of columnar cells (photocytes), characterized by the presence of coarse eosinophilic granules in the cytoplasm. The nervous supply of the elytrum derives from a nerve trunk which ascends the stalk or elytrophore and proceeds to a ganglion in the centre of the scale. From this ganglion nerves radiate peripherally and proceed to the photocytes, and to sensory receptors on the dorsal surface and at the margin of the scale (Bonhomme, 1942).

The normal response of a scale consists of a series of brief flashes, each about 80 msec. in duration, which appear over the entire photogenic area of the scale. The flashes usually start at a frequency of about 9/sec., but the rate soon falls off to about 1/sec., and is sometimes maintained for a minute. In rhythmic flashing of this pattern it is observed that the first few flashes gradually build up to maximal intensity, and then fall off in height. This progressive decrease in intensity of consecutive flashes is due to gradual exhaustion of luminescent material, and if the response proceeds long enough, the flashes ultimately become very weak, or disappear. When the response stops short of complete exhaustion, progressive fatigue can be produced by repeating the stimulation at suitable intervals.

Owing to the quick and repetitive character of the flashes, it has been possible to secure considerable quantitative data for physiological analysis of the response mechanism, which will be considered in the present paper.

Material and Methods

The luminescent responses of six species of polynoids occurring in the Plymouth fauna have been described previously, namely *Lagisca extenuata*, *Gattyana cirrosa*, *Harmothoë lunulata*, *Polynoë scolopendrina*, *Acholoë astericola* and *Malmgrenia castanea* (Nicol, 1953). Of these *Acholoë* and *Polynoë* proved most valuable for physiological study and were used extensively in the present investigation. The procedure has already been described. The scales were removed from the animals after narcotizing with magnesium chloride, and were stimulated electrically. Luminescent responses were recorded with a photomultiplier cell, electronic amplifier, and oscilloscope. Other procedures are described under the relevant sections.

Observations

The quick flashes which can be obtained by electrical stimulation of the scale are not affected in magnitude by variations in the strength of the stimulus. In scales which tend to flash rhythmically it has been found that a gradual increase in voltage has no effect until threshold is reached, when the scale starts to flash repeatedly at light intensities quickly rising to a maximum. Since such a response leads to exhaustion, it cannot be repeated in the same scale for the study of the effects of subsequent stimuli. Apparently, however, there is no gradual increment in response according to the strength of stimulus, but rather a maximal outburst when the effective strength is reached.

Although rhythmic flashing is the usual response to a single shock, occasionally an elytrum gives only a single flash to one stimulus. This is observed in elytra which have been stimulated previously by electric shocks and in some way fatigued, and also occurs in some elytra which have not yet been subjected to electrical stimulation. These latter preparations may well be from animals which have been excited during collecting and handling, or in which the elytral ganglion has been injured. It is difficult, however, to avoid contingencies of this kind. Preparations which will give only one flash per stimulus can also be secured by removal of the ganglion. This is described in a later section.

With scales that respond by a single flash to a stimulus it has been observed that once threshold is reached, further increase in stimulus strength brings about no increment in the intensity of the *quick* flash. This result is in agreement with the conclusion previously reached, that the luminescent flashes are produced by nervous stimulation, for excitation of the efferent nerve fibres is an all-or-nothing phenomenon.

When the stimulus strength is raised greatly, or the pulse duration is increased, a second type of response is obtained. This takes the form of a bright, prolonged glow, which quickly rises to a peak in about $\frac{1}{2}$ sec., and lasts up to 10 sec. (Fig. 1 A). Such a response frequently leads to complete exhaustion of the photogenic material contained in the scale, and subsequent stimuli are ineffective. In some preparations a pattern of rhythmic flashing is superimposed on the bright glow, or can be induced by repetitive stimulation (Fig. 1 B). It is concluded that in this type of response, partial or complete depolarization of the photocytes is achieved by direct stimulation of the latter, and that the effect of nervous impulses may intervene and influence the primary response. When scales which are being strongly stimulated are watched under the microscope it can be seen that the entire photogenic area lights up with a bright prolonged glow, or part of it does so, and rhythmic flashes proceed peripherally from a central glowing region. In the latter event only part of the photogenic area has been excited directly, and nervous stimulation is affecting the remainder of the photogenic field. For experimental purposes the strength of stimulation has been kept at a minimum in order to avoid direct excitation of the luminescent cells, but a certain amount of direct depolarization appears in a few of the records.

The Effect of Repetitive Stimulation

By using scales which respond initially by a single flash to a shock it is possible to analyse the effects of repetitive stimulation.

Single flash responses, one per stimulus, are illustrated in Fig. 1 C–E (*Acholoë*). The experiment recorded in Fig. 1 C, D will be considered in detail. It will be noticed that the overt responses to the first two stimuli (time interval 6 sec.) are barely perceptible (direct depolarization), but the response to the third stimulus is a definite flash. Continued stimulation, at a rate of 45/min. (interval 1·3 sec.) resulted in discrete flashes, one per stimulus, but after the fourth stimulus double flashes appeared, the beginning of repetitive flashing. All subsequent responses to a single stimulus consisted of several flashes. The responses also became much smaller with repetition, as the result of fatigue. A somewhat similar pattern is shown in Fig. 1 E. Here the response to the first stimulus was small (direct depolarization), the next six responses increased gradually in height, and occasionally there was a tendency for repetitive flashing.

The increase in height of successive responses with repetitive stimulation is a noteworthy feature of these records and is documented in Figs. 1 C–E and 3 D and in the accompanying graph (Fig. 2), based on a photographic record.

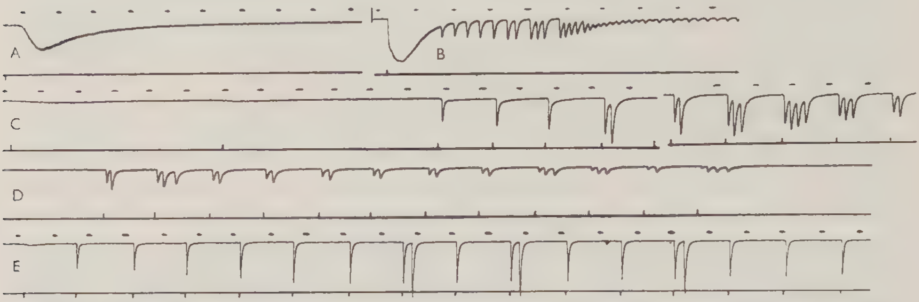

Fig. 1. A, protracted luminescent responses (elytrum of *Lagisca extenuata*), resulting from direct stimulation of the luminescent cells. Time scale, 1/sec. Effective stimulus, lower left. B, protracted luminescent response (elytrum of *Acholoë astericola*), on which are imposed rhythmic flashes after a delay of 1·8 msec. Time scale, 1/sec. Effective stimulus, lower left. C, D, single flashes and rhythmic flashing induced in an elytrum of *Acholoë* by single shocks and repetitive stimulation. Time scale above, 1/sec. Stimuli indicated on lower line. E, luminescent responses in an elytrum of *Acholoë*. Single flashes and repeated flashes induced by repeated shocks at a slow rate of 42/min. Time scale 1/sec. Stimuli shown on lower line.

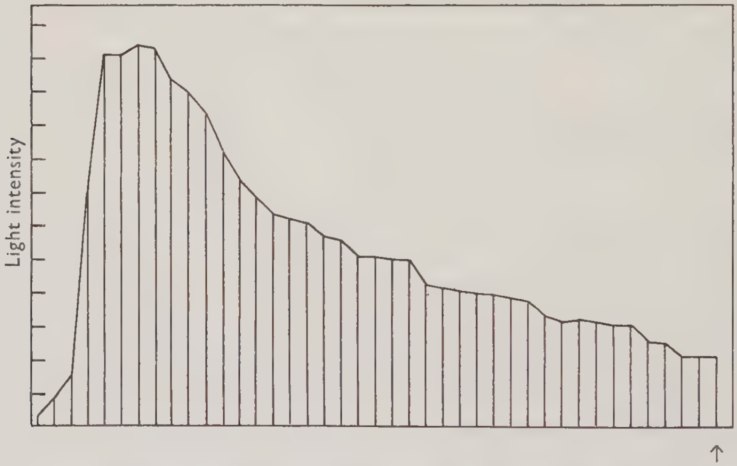

Fig. 2. A plot showing the change in intensity of successive responses in an elytrum of *Acholoë* stimulated by a burst of impulses at a frequency of 5/sec. Ordinates, light intensities in arbitrary units. Each vertical line represents a separate light flash. Arrow indicates end of stimulation.

Three factors are revealed in these records: (i) the response to the first stimulus may be small, but subsequent stimuli will evoke bright flashes; (ii) repetitive stimulation brings about an increase in the magnitude of successive flashes; (iii) finally, repetitive stimulation will evoke the onset of rhythmic flashes or flickering.

The effects obtained with repetitive stimulation can be compared advantageously with the course of events in rhythmic flashes evoked by a single stimulus. In the latter form of response it is also observed that the initial flashes increase in intensity (Nicol, 1953). Occasionally, when a stimulus produces only a single flash, two or more stimuli will start the scale flashing, again with progressive increment in light intensity (Fig. 3A–C, *Lagisca* and *Harmothoë*). Changes in the frequency of rhythmical flashing and the intensity of the flashes, however, are distinct phenomena, although the former is capable of influencing the latter.

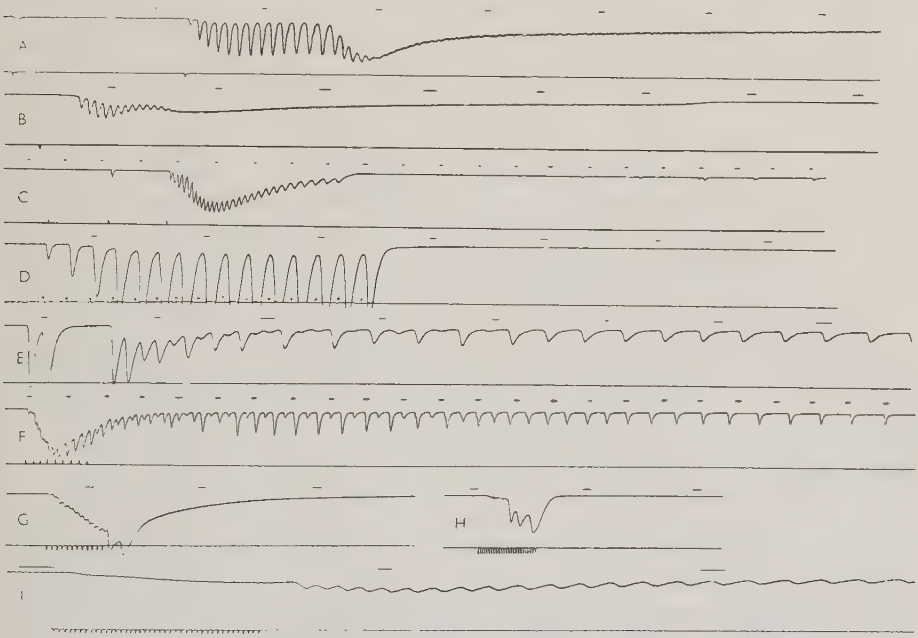

Fig. 3. A, luminescent responses of an elytrum of *Lagisca*, a rhythmic flashing induced by two consecutive impulses at an interval of 1·5 sec. B, flashing induced by a single stimulus. Time scale 1/sec. Stimuli shown on bottom line. The response in these records terminates in a prolonged glow. C, luminescent responses of an elytrum of *Harmothoë lunulata*. Repetitive flashing induced by three consecutive stimuli at a frequency of 42/min. Time scale, 1/sec. Stimuli shown on bottom line. D–I, luminescent responses of elytra of *Acholoë*, stimulated by series of impulses at different frequencies. Time scale above each record, 1/sec. Stimuli shown on lower line. Frequencies of stimulation are: D, E, 6/sec., E is a continuation of record D, and shows delayed rhythmic discharge; F, 6/sec.; G, 24/sec.; H, 36/sec.; I, 70/sec.

Summation of Luminescent Responses

When elytra are stimulated at low frequencies, below 7/sec., separate and distinct flashes are obtained (Figs. 1E, 3D, F). At frequencies somewhat greater than this the separate flashes begin to fuse together, and the degree of fusion becomes more and more pronounced at higher frequencies. In *Acholoë* the separate flashes are still perceptible at frequencies of about 25/sec. (Fig. 3 G), but are practically erased at frequencies reaching 40/sec. or more (Fig. 3 H, I). Similar results were obtained by stimulating isolated scales of *Polynoë* (Fig. 4 A, B), *Lagisca* (Fig. 4 C, D), and *Gattyana* (Fig. 4 E–H). Since the time taken to reach maximal intensity in a single flash is in the region of 40 msec. in *Acholoë*, it would be expected that a frequency of 25 or more impulses/sec. would be needed to bring about complete fusion of the separate responses.

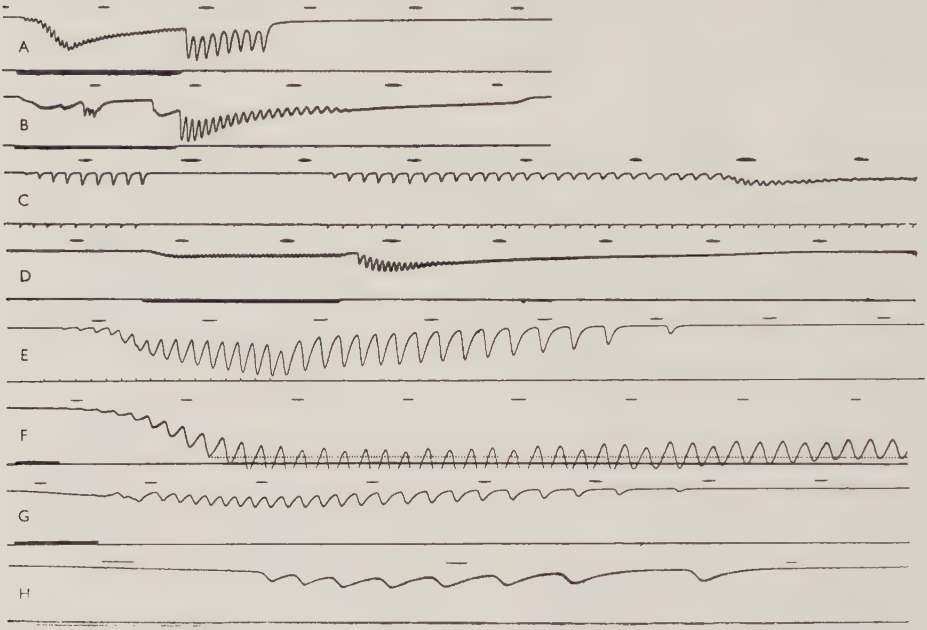

Fig. 4. A, B, luminescent flashes induced in isolated elytra of *Polynoë* by repetitive stimulation at 25/sec. (A), and 42/sec. (B). Time scale, 1/sec. C, repeated flashes in isolated elytra of *Lagisca* which were stimulated at 8/sec. (C), and 26/sec. (D). Time scale, 1/sec. E–H, luminescent responses of *Gattyana* elytra to repetitive stimulation. E, 8/sec.; F, 38/sec.; G, 66/sec.; H, 120/sec. Time scale above each record, 1/sec.

These results, which were produced by repetitive stimulation, again can be compared with records of protracted rhythmical flashing induced by one or a few stimuli. When rhythmic flashing is occurring at a slow rate (up to about 5/sec.), the separate responses are discrete and each one returns to base-line (zero intensity) (Fig. 3 A, B). When rhythmic flashing is taking place at higher rates than this, the separate flashes summate to various degrees, depending upon the intervals between consecutive flashes. For example, in

Fig. 3C (*Harmothoë*), rhythmic flashing shows a frequency of 9/sec. (interval 100 msec.), and there is a progressive build-up of intensity. Scales rarely flash rhythmically at a faster rate than this, which is insufficient to produce complete fusion.

If the frequency is gradually raised during a period of stimulation, the rate of flashing closely follows the rate of stimulation (Fig. 5A).

At high rates of stimulation the luminescent responses show irregularities in both frequency and amplitude. When the frequency is greater than 35/sec., the scale may not respond to every stimulus, but may begin flashing at some rate slower than the frequency of stimulation. In Fig. 5B (interval 21 msec.), for example, the scale shows initially a small smooth response, but ultimately begins flashing at a slow rate, much below the frequency of stimulation. During stimulation the scale responds either by discrete flashes, or a prolonged glow, according to the frequency, but once the stimulation ceases, the scale may continue to respond by rhythmical flashes at a slow frequency (Figs. 3G, I, 4A, B, E–H). That is, once the excitation at a fast rate is removed the now excited scale continues to respond normally at its own characteristic frequency. A third noteworthy feature is that the amplitude of the luminescent response is often small at higher frequencies of stimulation (either definite flashes or smooth response curve). Once stimulation has ceased, however, the

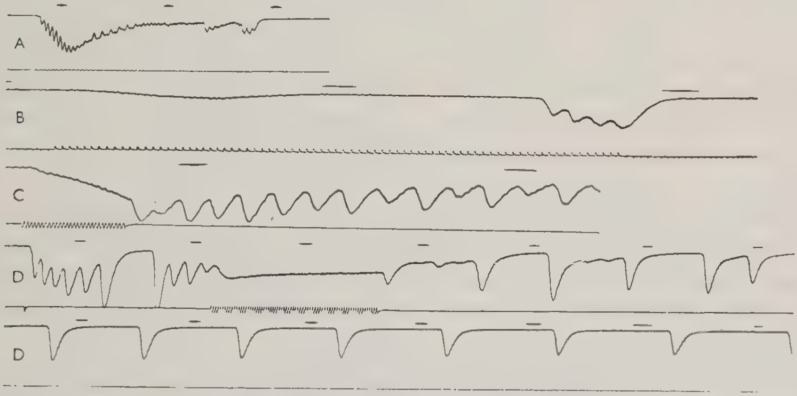

Fig. 5. A, increasing the frequency during a period of stimulation of the scale of *Acholoë*. The frequency of stimulation rises from an initial level of 28/sec. to 42/sec., and the flashes closely follow the rate of stimulation. Time scale above, 1/sec. B, repetitive stimulation (47/sec.) of an elytrum of *Acholoë*. There is initially a small smooth response, lasting 0·8 sec., followed by 4 flashes at a slow rate independent of the rate of stimulation. Time marks above, 1 sec. intervals. C, stimulation of an elytrum of *Acholoë* by alternating current at a frequency of 100 cycles/sec. During stimulation the luminescent response shows a slow rise, followed by rhythmic flashing at a slow rate (10/sec.) once electrical stimulation has ceased. Time marks above, 1 sec. interval. D, E, the effect of high-frequency stimulation on rhythmic flashing in an isolated elytrum of *Acholoë*. Rhythmic flashing was induced by a single impulse, and the scale was then stimulated by a burst of a.c. lasting 1·6 sec. at a frequency of 50 cyc./sec. Time scale above, 1/sec.

continued rhythmic responses of the scale often proceed at a much greater amplitude (Figs. 3G, 4A, B, F–H).

Several possible explanations can be advanced for these observations. The first is that stimulation at high frequencies produces polarization in the tissues, and is responsible for the small prolonged responses. This can be discounted since a.c. stimulation produces the same effects as condenser shocks (Fig. 5C). The second explanation is that the photocytes are supplied both by excitatory and inhibitory fibres, and that the effect of the latter predominates at high frequencies. This has been investigated by stimulating elytra into luminescence and then subjecting them to a short bursts of a.c. or condenser shocks at high frequencies during the period of rhythmic flashing. An example of these experiments is shown in Fig. 5D. Stimulation at high frequencies (above 50/sec.) indeed inhibits rhythmic flashing, which is resumed once stimulation has ceased, but a prolonged response, of greater or lesser intensity, continues during the period of high-frequency stimulation. The evidence accumulated is against the existence of inhibitory fibres. It has been noted above that responses may still occur at regular and slow frequencies during periods of fast stimulation (up to 150/sec.). Also a scale usually gives a prolonged smooth response during a period of rapid stimulation. The intensity of such a response is usually small, but in a small proportion of records it equals that of a normal flash. The postulation of inhibitory fibres seems unnecessarily complicated when considered in terms of the normal luminescent responses, which occur only on excitation and take place at a low frequency, and the mediation of such responses is probably effected solely by impulses in excitatory nerve fibres.

It is probable that these results can be best explained on the basis of a refractory period, either of the efferent nerve fibres, or of the photocytes. The latent period of the luminescent response is of the order of 31–21 msec. in *Polynoë* (Nicol, 1953), which includes the refractory period of the nerves. The refractory period of the luminescent response has been measured directly by arranging a Keith Lucas spring rheotome to deliver paired condenser shocks at suitable intervals. With this technique it has been determined that the refractory period lies between 9 and 16 msec., and a second impulse, falling within this interval, is ineffective. These figures give upper limits of 62–110 stimuli/sec. at which nervous stimulation would still be effective. At high frequencies, when successive impulses fall within the refractory period of the nerve, it would be expected that every second or third or subsequent stimulus would be effective, but such form of response has only occasionally been recorded, and that irregularly (Figs. 3H, 5B). Slight protracted responses (faint glow) have been obtained at frequencies as high as 150 pulses/sec., possibly resulting from some slight direct excitation of the photocytes, but actually the luminescent flashes usually show pronounced reduction in amplitude at frequencies from 20 to 40/sec. A peculiar feature is that even the response to the first stimulus, at high frequencies, is minute. It appears, therefore, that some terminal mechanism is incapable of responding fully or is blocked at high frequencies, and this effect may occur either at the nervous terminals, or in the photocytes themselves. Without a new approach, and additional data, the problem cannot be pursued further.

Interpretation of the Increment in Light Intensity

It has been ascertained that an increase in light intensity occurs during repetitive stimulation or during spontaneously rhythmical flashing. During prolonged stimulation and rhythmic flickering, however, the individual flashes gradually decrease in intensity and this is ascribed in part to fatigue of luminescent ability. The question is now posed whether the initial increase in the intensity of successive light flashes is due to summation of effector responses, or to some elevation in the level of excitation which in turn controls the level of the effector response.

It has been noted above that under rapid stimulation, or during rapid rhythmic flashing, the separate responses may follow each other so quickly that a new flash may begin before the previous flash is extinguished and recovery of that flash is completed (e.g. Figs. 3C and 4A). If the successive responses were of equal magnitude and each response were superimposed on the preceding one before the latter had time to return to zero, then a gradual build-up in light intensity should result. A similar result should obtain even if successive responses are of decreasing amplitude, so long as each falls during the rising phase of the inflexion or the early period of decay of the preceding response curve. The arithmetic relations are sufficiently obvious not to need further elaboration. This will partly explain the progressive increment in light intensity which can be seen in many of the records.

In these records of repeated responses, however, it is apparent that another factor is operating in addition to summation of the light intensities of separate flashes. Often the response to the first stimulus is very small, and subsequent responses are many times greater than the initial response, and increase rapidly in magnitude. Also, the peaks of the first few responses may show a stepwise increase in intensity which is too great to be due entirely to partial summation of individual responses. When the light intensities of the first few responses are measured from the beginning of the inflexion to the peak of each flash, it is frequently found that a given response is greater than the one immediately preceding it.

When the responses are occurring at a slow rate (either rhythmic flashing, or as the result of continued stimulation at a low frequency), separate flashes may be observed, each of which decays to zero before another begins (Figs. 1C, D, 3A, B, D, E). Under these conditions, as previously indicated, the initial few responses increase successively in height.

The relative amount of increment shown by successive responses varies considerably from specimen to specimen, and of course changes in the same specimen according to its physiological condition and the rate of flashing. When the initial response is large, the following responses increase gradually and progressively in height, but when the initial response is minute, then the increase of intensity in the succeeding first few responses is very striking (Figs. 1C, D; 3A, B, D, E; 4E).

It is concluded from these results that each stimulus establishes a protracted state of facilitation, and, when the successive stimuli are arriving at suitable intervals, the corresponding facilitatory states are additive and heightened excitation results. This in turn controls the intensity of the response up to a certain limit determined by the physico-chemical conditions of the luminescent reaction. The effective duration of the facilitatory state is remarkably long, and it has been possible to characterize it as follows.

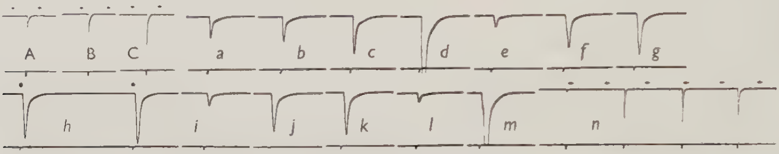

Fig. 6. A–C, single flashes from an elytrum of *Polynoë* stimulated at various intervals. Stimuli, single condenser shocks. A, initial flash; B, second flash after an interval of 4 min; C, third flash after an interval of 3 min. Time scale, 1/sec. *a–n*, the effect of paired stimuli delivered at various intervals in scales of *Polynoë* which responded by giving a single flash per stimulus. Frames *a* to *m* from a single scale; *n* from another scale. Time intervals between stimuli in consecutive frames as follows: *a–b*, 2 min.; *b–c*, 1 min.; *c–d*, 30 sec.; *d–e*, 2 min.; *e–f*, 15 sec.; *f–g*, 15 sec.; *g–h*, 15 sec.; *h–i*, 2 min.; *i–j*, 10 sec.; *j–k*, 10 sec.; *k–l*, 2 min.; *l–m*, 1 min. The two stimuli in *h* were separated by an interval of 1 sec.; the consecutive stimuli in *n* were separated by intervals of 1·5 sec. Time scale, 1 sec., for frames *a* to *m* shown in frame *h*; time scale for frame *n*, 1/sec., above.

Duration of the Facilitatory State of the Luminescent Response

In a previous communication (Nicol, 1953, fig. 12) it has been shown that the effect of a previous stimulus, as revealed in the increase of the light intensity of a succeeding flash, may last as long as 6 sec. in *Acholoë*. A detailed study of this phenomenon is documented in Fig. 6 from records of single flashes in *Polynoë scolopendrina*. In a fresh preparation subjected to repeated stimuli at various intervals, the effect of the first of two stimuli lasts as long as 4 min. (Fig. 6A–C). After this interval, a second stimulus gives a measurably larger response, amounting to an increment of 33% (Fig. 6B). When a scale has been repeatedly stimulated and fatigue sets in, the response elicited after several minutes shows no increment, or may even be slightly less than a previous flash (cf. *a*, *b*, and *d*, *e*, in Fig. 6). This could be due to fatigue in the system of neuro-effector transmission, but it is simpler to regard it, tentatively, as resulting from progressive exhaustion of luminescent material. The assumption is made that the intensity of a response is a function of the level of excitation and the quantity of luminescent material available in the photocytes. Since the residual facilitation after a protracted interval is small, the heightened response which it would be expected to provoke is cancelled by the concomitant decrease in luminescent material available for the manifestation of the response.

As the interval between two consecutive impulses is shortened, the second response increases relative to the first response in magnitude (Fig. 6, *b–k*). This is shown with special clarity in frame *n* (stimulation interval, 1·5 sec.). In the pair of records shown in frames *l* and *m*, the second stimulus set off a double flash, with an interval of about 30 msec. between the two components, and with this interval the second peak of the double flash showed an increment of over 300%. Data from a scale have been plotted in Fig. 7, which shows that the excitatory effect of a previous stimulus decreases with time, and that the rate of decay of facilitation or excitation follows an exponential curve. If the material basis of this facilitation be thought of as a substance released or liberated at or in the effector cells by the arrival of a nervous impulse, then the curve of decay of facilitation shown in Fig. 7 suggests a simple monomolecular reaction effecting the removal of the substance concerned and converting it into an inactive form. On the same basis, the increment of excitation in successive responses, at brief intervals, as recorded by the rise in intensity of sequential flashes, would be due to gradual accumulation of an excitatory or complementary substance at a rate faster than it could be removed.

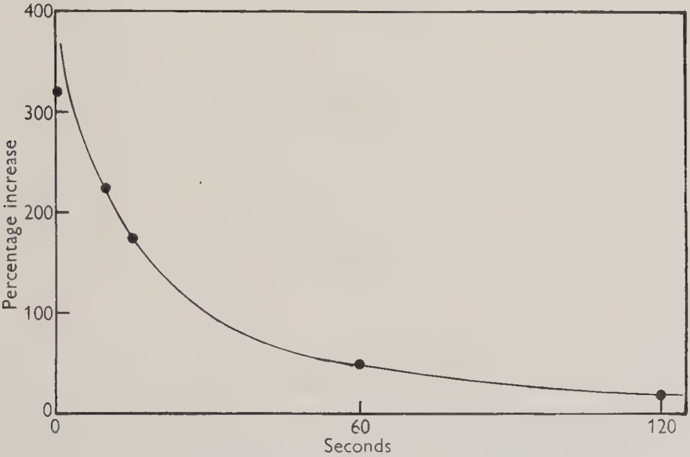

Fig. 7. A curve based on measurements of the light intensity produced by the second of a pair of flashes in a series in which the paired stimuli were delivered at varying intervals. Percentage increment in the intensity of the second response over the first is plotted against time between the two stimuli (sec.) Elytrum of *Polynoë*.

Rhythmic Flashing

In very excitable preparations a single shock initiates prolonged rhythmic flashing (see Nicol, 1953, for records of typical responses). Other scales give only a single flash per stimulus, and there are intermediate cases in which a single stimulus gives rise to a few flashes, or which require a number of impulses to start rhythmic flashing (Figs. 1 C, D; 3 A, B, C).

From an examination of the many records collected from different animals, it became apparent that the onset and duration of rhythmic flashing are dependent on the previous history and the excitatory state of the scale. It also appeared that rhythmic flashing shows a condition of progressive fatigue akin to that revealed in diminution of intensity during the course of repeated flashing. An attempt has been made to analyse the factors involved as follows.

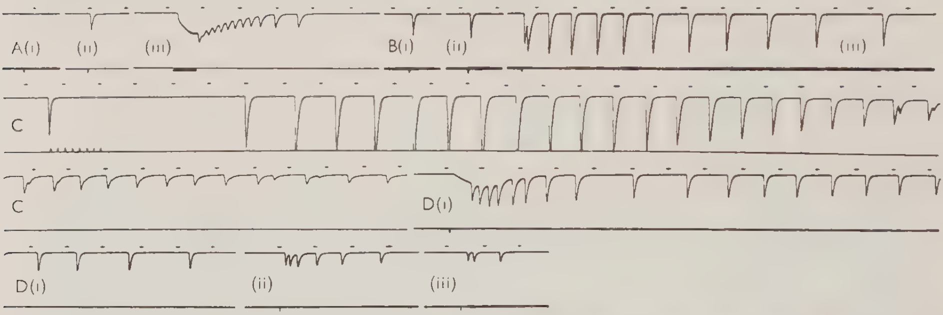

Fig. 8. Luminescent responses of scales of *Acholoë*. The effects of single stimuli and repeated stimulation on rhythmic flashing. Time scale in all records, 1/sec. A, single pulses in (i) and (ii) followed by a burst in (iii) (0·75 sec. at 22/sec.). 12 sec. between (i)–(ii), and (ii)–(iii). B, single pulses in (i) and (ii) at an interval of 10 sec., showing increment in light intensity, followed by a third pulse after an interval of 1·6 sec. in (iii), which resulted in prolonged rhythmic flashing. C, rhythmic flashing induced by a burst of impulses, with a pause of 5 sec. between the first and succeeding flashes. D, reduction in the number of flashes in consecutive periods of response. Stimulation, a single pulse in each of frames (i), (ii) and (iii).

In preparations which respond by rhythmic flashes, there are sometimes long intervals, extending to 15 sec., between consecutive flashes or groups of flashes (Figs. 3 D, E; 8 C). In these cases the scale suddenly starts to flash rhythmically, often at a fast rate, several seconds after the response apparently has ceased. This indicates that the residual excitatory condition connected with the rhythmicity of the response can last for surprisingly long periods.

When a single pulse fails to evoke rhythmic flashing, several pulses or a burst of pulses will frequently do so. The excitatory condition responsible for rhythmic discharge, consequently, can be influenced by repetitive stimulation. The level of underlying excitation is augmented by repeated stimulation, resulting in an increase in the *number* of rhythmic flashes which occur after the stimulus or period of stimulation has terminated (Figs. 1 C, D; 8 A, B).

In an attempt to determine how long a stimulus will continue to influence the onset of rhythmic flashing, suitable scales were stimulated by pulses separated by intervals extending up to 2 min. Rhythmic flashing was evoked by stimulating at frequencies as slow as 1 per 12 sec. As noted above, rhythmic flashing has appeared spontaneously after quiescent periods of up to 15 sec. after a previous display has subsided. My data, therefore, set a limit of 12–15 sec. for maximal duration of the effective excitatory state governing the onset of rhythmic flashing.

Finally, it has been observed that when a scale is stimulated on successive occasions into rhythmic flashing, each successive period of rhythmic flashing is of shorter duration, until eventually only one flash results from a stimulus, and a burst of impulses is then necessary to evoke a rhythmic discharge. A gradual fatigue or diminution of excitatory potentiality thus takes place with repetition, and this is manifested as a reduction in the number of flashes resulting from a single stimulus or given number of stimuli (Fig. 8D).

The presence of a ganglion in the elytrum suggested that this structure might be responsible for the rhythmic character of the flashing observed in severed scales. Experiments to test this hypothesis were carried out as follows. Elytra were narcotized with $MgCl_2$, and were cut into two under a dissecting microscope in such a way that one half of each scale contained the elytrophore stalk and ganglion, and the other lacked this structure. The preparations were then washed out in sea water and the two halves of each scale were stimulated separately. Specimens of *Acholoë* and *Polynoë* were used in these experiments. With a long series of bisected scales it was observed that half scales containing a ganglion responded to a single shock by a series of rhythmic flashes or a single flash, whereas half scales lacking a ganglion never gave rhythmic flash responses (Fig. 9A). It is concluded that the elytral ganglion is involved in mediating the rhythmical flashing which is the characteristic response of normal elytra.

The occurrence of continued and rhythmic flashing long after the effective stimulus (tactile or electrical) has ceased denotes the existence of some repetitive excitatory mechanism governing its evocation. From several lines of evidence it is possible to cull the more likely explanations of this phenomenon, and to outline its main features. This evidence points towards repetitive discharge in nerve cells as the responsible factor involved.

Rhythmic flashing is not due to the recurrence of some excitatory condition at or in the peripheral effector cells for the following reasons. In the majority of records the flashes show great regularity in their timing and characteristics, each flash consisting of a smooth 'unimodal' curve, and subsequent flashes appearing at regular intervals. It seems highly improbable that a mass of isolated units (glandular cells) could maintain a rhythm of cyclical activity, as the result of an initial triggering stimulus, so as to produce such a series of discrete and uniform synchronous responses. The possibility of the light flash from one cell or group of cells affecting the others in turn, and so cyclically, is ruled out by the fact that the luminescent cells are not sensitive to illumination.

The crucial experiment consists of recording from fragments of elytra. As reported in the previous section, scale fragments lacking the ganglion give only a single flash to each electrical stimulus, whereas fragments containing the elytral ganglion flash rhythmically when subjected to a single stimulus. This ganglion lies in the centre of the elytrum and contains a number of nerve cells. It receives a nerve trunk from the elytrophore and gives rise to nerve fibres which radiate out over the elytrum and proceed to the photocytes.

Continued flashing might be due to repetitive firing in nerve fibres supplying the luminescent cells, but the fact that a fragment of a scale lacking the ganglion, but still retaining its peripheral nerve fibres, fails to flash repetitively, rules this out. The evidence thus implicates the ganglion cells in the control of rhythmic flashing.

Without some more direct method of recording, the exact mechanism must remain a subject for speculation. The rhythmic flashing may be due to regular oscillations in the excitatory state of nerve cells lying in the elytral ganglion. According to this hypothesis the excitatory oscillations would be initiated by some stimulus (electrical, nervous, mechanical), and nerve impulses would be generated in the efferent fibres at the excitatory peaks of the cycle. A second hypothesis assumes the existence of oscillatory nervous arcs whereby nervous impulses can continue firing in closed circumscribed circuits within the ganglion, and in this central locus each circular sweep of impulses fires the efferent nerve trunks. The process involves a very interesting instance of rhythmic nervous activity in a peripheral ganglion at a low phyletic level, and is worthy of further investigation.

The flashes often appear uniform enough to represent synchronous or nearly synchronous activation of all the photogenic cells in the scale. Occasionally there is a slight wobble in the response spikes which may represent slight differences in the timing of nervous impulses arriving in different regions of the scale. A uniform and regular pattern of flashing is the usual form of response in the scales of all species, but occasionally there is asymmetry in the pattern of rhythmic flashing. Sometimes there are alternate periods of strong and weak flashes, at other times the pattern is more irregular, with variable periods between spikes of different amplitude (Figs. 3D, E; 9B). It has been suggested previously that these asymmetrical responses are due to the separate activity of two or more neuro-effector units firing at different rates and acting out of phase, so that responses at one time are distinctly separable, and at others partially or wholly summated (Nicol, 1953).

The Effect of Drugs on the Luminescent Response

Drug action has been explored in a number of luminescent species, and in view of the peculiar conditions of nervous regulation obtaining in the luminescent responses of polynoids, it seemed desirable to determine whether the action of certain drugs would throw any light on the processes involved. The following drugs were tested: adrenaline, acetylcholine, nicotine, eserine, atropine, curare, and strychnine. These had little or no effect on the luminescent responses of isolated scales, as the following summaries show.

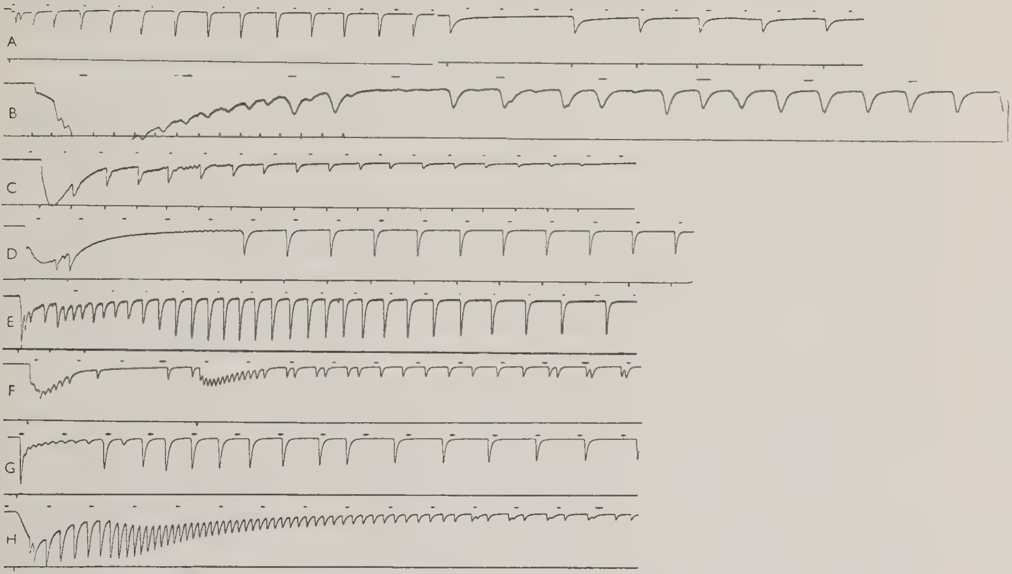

Fig. 9. A, stimulation of half scales of *Acholoë*. Left, rhythmic flashes in part of an elytrum containing a ganglion. Stimulation consisted of a single pulse. Right, single flashes, one per stimulus in the other portion of the same elytrum. Time scale, 1/sec. B, rhythmic flashing induced in an elytrum of *Acholoë* by a burst of impulses. Time scale, 1/sec. C–H, effects of drugs on the luminescent responses of isolated scales of *Acholoë*. Scales were immersed in solutions of the drugs for the times stated and then subjected to electrical stimulation (single or repeated shocks). Time scale, 1/sec. C, adrenaline 1/10,000, 20 min.; D, acetylcholine 1/10,000, 120 min.; E, nicotine 1/100,000, 70 min.; F, eserine 1/100,000, 50 min.; G, atropine 1/100,000, 3 hr.; H, D-tubocurarine, 1/10,000, 30 min.

Adrenaline. Concentrations, 1/1,000,000 to 1/10,000 for 30–60 min. This drug failed to excite isolated scales, and when treated scales were stimulated electrically, they still responded rhythmically and gave bright flashes very similar to those produced by normal untreated preparations (*Acholoë*) (Fig. 9C).

Acetylcholine. Concentrations, 1/1,000,000 to 1/1,000 for $\frac{1}{2}$–$2\frac{1}{2}$ hr. Solutions of the highest concentrations were adjusted to pH 8·2 with sodium acetate and sodium hydroxide. This drug failed to induce luminescence in isolated scales. At concentrations of 1/1,000,000 to 1/10,000, scales still gave bright flashes and repetitive discharges when subjected to electrical stimulation, and the response recorded appeared normal when compared with those of untreated scales. At a concentration of 1/1000, some scales failed to respond, or gave reduced responses (*Acholoë*, *Polynoë*) (Fig. 9D).

Nicotine. Concentrations of 1/1,000,000 to 1/1000 for 30–75 min. Scales did not flash when placed in solutions of nicotine. Electrical stimulation of treated scales produced responses, single bright flashes and rhythmic flashes, entirely similar to those of untreated scales (*Acholoë*, *Lagisca*) (Fig. 9E).

Eserine. Concentrations, 1/1,000,000 to 1/10,000 for 30–60 min. Scales placed in solutions of eserine did not luminesce and electrical stimulation was employed to determine whether the drug had any effect on the characteristics of the response. At all strengths the scales still responded with bright flashes and rhythmic discharges. Eserine, consequently, does not produce block, and neither does it prevent the appearance of bright flashes (*Lagisca*, *Acholoë*) (Fig. 9F).

Atropine. Concentrations, 1/1,000,000 to 1/1000, up to 3 hr. This drug was without effect on the luminous response. Rhythmic bright flashes were still obtained on electrical stimulation of the scales after immersion in atropine solutions, and these appeared in no wise different from those of normal untreated scales (*Acholoë*) (Fig. 9G).

Curare (D-*tubocurarine*). Concentrations, 1/1,000,000 to 1/10,000 for 30–60 min. This drug had no apparent effect. Bright flashes and rhythmic flashing, in no way different from the responses of untreated scales, were obtained on electrical stimulation of scales treated with D-tubocurarine (*Acholoë*) (Fig. 9H).

Strychnine sulphate. Concentrations, 1/1,000,000 to 1/1000, for 30 min. Strychnine was tried because of its well-known excitatory effect on nerve cells in the central nervous system of vertebrates. Since the rhythmic flashing of polynoid scales is due to some kind of repetitive discharge from ganglionic cells in the scale, it was considered worth while to determine whether strychnine would affect these nerve cells in a manner similar to its effects on vertebrate neurones.

Strychnine, in the above concentrations, did not cause the scales to flash. At a concentration of 1/1000, however, it did abolish the response to electrical stimulation. The effect was reversible in that after washing out the drug, the scale could be induced to flash normally under electrical stimulation (*Polynoë*, *Acholoë*, *Gattyana*).

It is noteworthy that none of these drugs, with the exception of strychnine, was effective in abolishing the luminescent flashes which can be induced by electrical stimulation. Since the quick flash-response is due to nervous excitation, it seems reasonably certain that atropine and curare are unable to block neuro-glandular transmission in the scales of these animals. Neither acetylcholine nor adrenaline was effective in inducing a luminous response in the scales, and this would appear to exclude these substances as excitatory agents. Adrenaline is ruled out as an inhibitory agent since normal luminescent responses can still be elicited after its application; and there is no evidence that acetylcholine and eserine had any pronounced depolarizing action, in view of the quick flashes which attended electrical stimulation of scales which were treated with these substances. Strychnine, in high concentrations, is known to block axon conduction, and in the present experiments its inhibitory effect was probably achieved through this route (Heinbecker & Bartley, 1939; Coppée & Coppée-Bolly, 1941).

The effect of pharmacological agents on luminescent tissues has been explored to only a very limited extent, and comparative data are fragmentary. Adrenaline fails to elicit luminescence in the polychaete *Chaetopterus* (Nicol, 1952*a*), and in the decapod crustacean *Systellaspis* (Harvey, 1952). It apparently decreases the sensitivity to mechanical stimulation in the ctenophore *Mnemiopsis* (Chace, 1941). There is no evidence from any other source that adrenaline is normally produced in these animals, and this may be correlated with the absence of any pronounced physiological action. Chromaffine cells (believed to secrete adrenaline) have been described in the Aphroditidae (in *Aphrodite aculeata*), however, but the present evidence shows that adrenaline is not involved in the luminescent responses of polynoids (Gaskell, 1914). In contrast, adrenaline forms a powerful stimulant to light production in fire-flies (Coleoptera). The action appears to be an indirect one, on the tracheae and cells, and not direct on the luminescent cells. In consequence, the tracheoles are dilated and the photogenic cells receive a greater supply of oxygen, which in turn regulates the oxidative luminescent process and the intensity of light emitted. Since adrenaline, so far as known, does not occur naturally in insects, this effect appears to depend on fortuitous sensitivity to adrenaline (Creighton, 1926; Emerson & Emerson, 1941).

Quite different is the situation in teleosts where adrenaline is normally produced by suprarenal tissue, and shows a sympatheticomimetic effect on many visceral activities (Nicol, 1952*c*). Injections of adrenaline into *Porichthys notatus* and *Echiostoma ctenobarba*, two species of teleosts bearing photophores, cause them to luminesce (Greene & Greene, 1924; Harvey, 1931, 1952). This is suggestive evidence that adrenaline, which is a blood-borne hormone, may normally be involved as a chemical mediator as well in the luminescent response of these species. It is not unlikely that the serially arranged photophores in the head and trunk of teleosts are innervated by post-ganglionic neurones of the sympathetic nervous system, via recurrent grey rami, and cranial and spinal nerves. Branches of these nerves are known to innervate photophores in *Argyropelecus* and *Lampanyctus*, but fibre-pathways have not been worked out (Handrick, 1901; Ray, 1950). At least the positive response to adrenaline suggests that nerve fibres supplying the photophores may be adrenergic in nature (Nicol, 1952*c*).

Turning now to acetylcholine and other parasympatheticomimetic drugs, we find much less information available. Both acetylcholine and nicotine evoke luminescence in *Chaetopterus*, and the response to electrical stimulation is augmented by eserine (Nicol, 1952*a*, *b*). In *Mnemiopsis*, Chace (1941) found that eserine increases the sensitivity to mechanical stimulation, and also increases the duration of the luminescent flashes. Acetylcholine, in high concentrations (1 in 3000), enhances this effect. Muscarine and pilocarpine (1 % solutions in sea water) have a strong excitatory effect on the luminescent responses of *Ophiopsila annulosa* (Ophiuroidea). Atropine appears to have an inhibitory effect, as judged by responses to mechanical stimulation

(Mangold, 1907). Luminescence in the millipede *Luminodesmus sequoiae* is not inhibited by curare, but there is no evidence that light production is under nervous control in this species (Davenport, Wootton & Cushing, 1952). During a cruise on the R.R.S. *Discovery II* in 1952, I have ascertained that neither *Pelagia* nor *Systellaspis* lights up after treatment with acetylcholine in various concentrations (10^{-6} to 10^{-4}).

I have collected these scattered observations dealing with the effects of drugs on luminescent responses in order to expose any similarities and trends which may exist. There is obviously much variation in the effects of the several drugs listed above on different species. This is probably to be expected on several grounds. Luminescence, as one type or category of effector response, is often equated with others such as muscular contraction, chromatophore activity, glandular secretion, etc. The mechanisms of luminescence are markedly different in various species, however; indeed, several different mechanisms sometimes co-exist in the same species. In some species a luminescent secretion may be expelled, in others light is produced as the result of intracellular oxidative processes. In still other species the light is continuous, and muscular mechanisms or chromatophore-screens control the emission. With this heterogeneity of mechanisms it is not surprising that external agents should have extremely diversified effects on different luminous organisms. These several mechanisms are in turn regulated by the nervous system, and the details of innervation vary with the morphological complexity of the animal and the character of the tissue innervated. The effects of various pharmacological agents on the nervous systems of different animals are extremely varied, and this is reflected in the luminous responses. In evaluating the significance of drug action on the luminescent responses of any given species, it is advantageous to know the effects of a particular drug on other effector tissues and on the nervous system of that organism. Evidence from related fields of physiology and morphology may then indicate certain excitatory and effector processes which may be operative in the organism under investigation, and which can be further tested in terms of the luminescent response. Investigations of this kind have rarely been attempted.

The Effect of Cyanide

Luminescence is basically an oxidative reaction, and depends on oxygen. For this reason the effect of cyanide, a respiratory inhibitor, was tried on isolated scales (*Acholoë*, *Harmothoë*). KCN, in concentration 0·0001 M for 1 hr. failed to prevent luminescence following electrical stimulation. In concentration 0·001 M, KCN abolished luminescence in 45–60 min. It seems probable, from the concentrations and times required to abolish the luminescent response, that KCN is acting by slow poisoning of efferent nerves. Cyanides, generally, have little effect on luminescent responses and reactions of animals (see Harvey, 1952).

The Effect of Unbalanced Salt Solutions

In order to test the effect of different ions on the luminescent responses of polynoids, isolated scales were immersed in isosmotic solutions of selected salts. All experiments were carried out with scales of *Lagisca*, *Acholoë*, and *Gattyana*. At least four elytra were tested in each solution. The pH was adjusted to 8·2 with cresol red, unless otherwise stated.

Isotonic salt solutions having the following salt concentrations were used.

	g./l.		g./l.
NaCl	31·56	$MgCl_2$	73·20
KCl	40·26	Choline chloride	65·64
$CaCl_2$	39·95		

Freshwater. When dropped into distilled water, scales invariably luminesce. This takes the form of either a prolonged glow, or repeated flashing.

Solutions of simple salts

KCl. Isosmotic solutions of KCl always produce a bright prolonged glow which persists until the scale is exhausted.

NaCl. In solutions of isosmotic NaCl, scales respond by slow or rapid repetitive flashes, which continue for some time and are followed by a steady glow.

$CaCl_2$ (pH 7·9–8·2). There is some doubt about the effect of this salt. In a few scales it evoked an initial brief flash, followed by repetitive flashing, but in the majority of scales its application was not attended by a luminescent response. It appeared to enhance the sensitivity of scales, however, since these were easily excited into luminescence by slight mechanical agitation after being placed in a solution of $CaCl_2$.

$MgCl_2$. No apparent effect.

Mixtures of isotonic salt solutions

The proportions of the different salt solutions are given in volumes.

NaCl 25 + *KCl* 0·6. Scales responded by rhythmic flashing.

NaCl 25 + $CaCl_2$ 1. No response observed.

NaCl 25 + $MgCl_2$ 5. No response observed.

NaCl 25 + *KCl* 0·6 + $CaCl_2$ 1. No response observed.

NaCl 25 + *KCl* 0·6 + $MgCl_2$ 5. No response observed.

NaCl 25 + *KCl* 0·6 + $CaCl_2$ 1 + $MgCl_2$ 5. No response observed.

KCl 25 + $CaCl_2$ 0·6. Scales responded by a bright prolonged glow.

KCl 25 + $MgCl_2$ 5. Scales responded by a bright prolonged glow.

KCl 25 + $CaCl_2$ 0·6 + $MgCl_2$ 5. Scales responded by a bright prolonged glow.

$MgCl_2$ 25 + $CaCl_2$ 1. No response.

Mixtures of isosmotic salt solutions and choline chloride

Choline chloride was employed as a physiologically inert substance to replace some one or other salts in the test solution.

Choline chloride. This substance by itself produced no luminescent response.

NaCl 75 + *choline chloride* 25. Scales flashed rhythmically in this solution.

KCl 2 + *choline chloride* 98. No response observed.

KCl 10 + *choline chloride* 90. Scales responded with a quick flash followed by a prolonged glow.

$CaCl_2$ 3 + *choline chloride* 97. In some preparations occasional weak flashes were observed after a considerable interval, but the majority of scales gave no overt response.

NaCl 75 + *KCl* 2 + *choline chloride* 23. This solution evoked a few quick flashes followed by a prolonged glow.

NaCl 75 + $CaCl_2$ 3 + *choline chloride* 22. This solution evoked slow rhythmic flashing.

NaCl 75 + $MgCl_2$ 15 + *choline chloride* 10. No response observed.

NaCl 75 + $CaCl_2$ 3 + $MgCl_2$ 15 + *choline chloride* 7. No luminescent response observed.

KCl 2 + $CaCl_2$ 3 + *choline chloride* 95. One scale out of six gave rhythmic flashes.

KCl 2 + $MgCl_2$ 15 + *choline chloride* 83. No response observed.

KCl 2 + $CaCl_2$ 3 + $MgCl_2$ 15 + *choline chloride* 80. A faint persistent light was observed.

NaCl 75 + *KCl* 2 + $CaCl_2$ 3 + *choline chloride* 20. No light observed.

NaCl 75 + *KCl* 2 + $MgCl_2$ 15 + *choline chloride* 8. No light observed.

$CaCl_2$ 3 + $MgCl_2$ 15 + *choline chloride* 82. No observable response.

These responses may be summarized by observing that KCl in excess (4 g./l. or more) produces a prolonged steady glow; NaCl in amount equivalent to that occurring in sea water, evokes rhythmic flashing; $CaCl_2$ has little effect by itself; and $MgCl_2$ acts as an anaesthetic. In a mixture of NaCl and KCl the scales respond initially by rhythmic flashes and then give a prolonged glow. The addition of Mg abolishes the rhythmic flashing called forth by Na, but not the prolonged glow due to K. Ca likewise reduces or abolishes the effect of Na, but not of K, at least in excess. As is usually found in experiments of this kind, the addition of further ions restores the balance; thus, neither Na + K + Ca nor Na + K + Mg evokes a luminescent response.

The depressant effect of Mg on excitable tissues is well known, and its anaesthetic action calls for no further comment here. Ca and Mg individually, and together, act as ionic antagonists for Na, and either opposes the excitable effects of Na + K. Antagonistic effects of this kind on excitable tissues have been frequently observed among marine invertebrates, and many examples can be found in research publications.

In an investigation of the effects of cations or combinations of cations, two apparent factors are involved, viz. the ability of the cations to excite tissues, and the ability to modify excitability as tested by stimulation. With the exception of Mg ion, only the former action has been tested in the present experiments. From the nature of the effects produced, certain conclusions can be drawn. K ion, in excess, is known to have a strong depolarizing effect on excitable tissue (nerve, muscle), and the prolonged glow which is the result of immersing elytra in an isosmotic solution of K is evidence of a direct depolarizing effect on the photocytes (Hodgkin & Huxley, 1945; Calma & Wright, 1947). In effect, it is very similar to the bright prolonged response which follows strong stimulation of a scale, also ascribed to direct excitation of the luminescent cells.

Sodium ion also has a stimulatory effect on nerve, and its role in the transmission of the nervous impulse has recently been discussed by Hodgkin (1951).

In the present experiments it is noteworthy that Na, initially at least, acts on the nerve and not directly on the photocytes, as revealed by the rhythmic discharge which its application elicits. The depolarizing action of K on the photocytes is antagonized by Na, and these two ions together stimulate the efferent neurones, producing rhythmic discharge and flashing. Neither Ca nor Mg, it may be noted, counteract the depolarizing effect of K (0·52–0·43 M) on the photocytes, but each is effective in reducing or preventing the stimulatory effect of Na ion on the efferent neurones supplying the photocytes.

Different cations have been tested on a variety of luminescent metazoans, and many scattered observations are now available, by no means easy to interpret. The scyphomedusan *Pelagia noctiluca* becomes luminescent in a solution of KCl, and, on poisoning with Ca, spontaneous luminescence appears over the whole surface. In the absence of Mg, i.e. in a solution of Na, K and Ca, *Pelagia* passes into a state of hyperirritability, and flashes of light appear on the bell (Heymans & Moore, 1923, 1924). The pennatulid *Cavernularia habereri* luminesces in isosmotic KCl but not in NaCl. Moreover, luminescence is evoked by all solutions containing K, and combinations of K plus one, two, or three other salts, viz. NaCl, $CaCl_2$ and $MgCl_2$. The other three ions, in the absence of K, fail to induce luminescence. It is rather surprising that a solution containing $NaCl+KCl+CaCl_2+MgCl_2$ should evoke luminescence. Since equal quantities of isosmotic solutions of the four salts were used in making up the test solution, the resultant concentration of K would be *c.* 14 times that in sea water, and the excitatory effect produced could be ascribed to K in excess. Unfortunately, the hydrogen-ion concentrations of the salt solutions do not appear to have been regulated, and the relatively high acidities of the unbuffered salts may themselves have produced excitatory effects (King-Li-Pin, Tchang-Si, Tai-Lee & Liu-Yu-Su, 1936). It is reasonable to conclude that K has a similar stimulatory effect on *Cavernularia* as on *Pelagia*, but whether on the nervous system, or directly on the photogenic cells, is unknown. The ctenophores *Mnemiopsis* and *Beroë* also luminesce when treated with isosmotic solutions of KCl and $CaCl_2$, but not NaCl and $MgCl_2$, results similar to those obtained with *Pelagia* (Moore, 1925).

In *Chaetopterus variopedatus*, a luminescent polychaete, isosmotic KCl causes bright luminescence, and NaCl, though still effective, produces a fainter glow. *Chaetopterus* is a species which discharges a luminescent secretion by some contractile process, and it is not unlikely that K produces its effect by exciting the glandular cells directly. In this animal Ca and Mg fail to inhibit excitation by K, but they reduce or abolish the stimulatory effect of Na, possibly by blocking Na depolarization of efferent nerve fibres supplying the photocytes. When Ca or Mg is added to a solution of NaCl and KCl, luminescence fails to appear, or is greatly reduced (Nicol, 1952*a*).

Some additional data are available. Mangold (1907) observed that isolated spines of *Ophiopsila annulosa* will luminesce in strong NaCl solutions. Shōji

(1919), who studied the effects of ions on the luminescence of the squid *Watasenia scintillans*, was concerned with the persistence of luminescence in the mantle-photophores, and retention of irritability to stimulation. His tables indicate that luminescence and irritability persist for about the same length of time in solutions of NaCl, KCl, and $CaCl_2$, but several times longer in solutions of $MgCl_2$. These solutions were osmotically equivalent to one another but only one half of the values for sea water ($\Delta - 0{\cdot}93^\circ$ C.). It is not clear in these experiments to what extent the several ions initially excited luminescence. With combinations of salts it appears that Mg is most favourable for continued luminescence. Luminescence and irritability persisted longest in combinations lacking Ca (i.e. Na + K + Mg). The photophores soon ceased to glow in isosmotic solutions containing Ca (i.e. Na + K + Ca), but continued to respond to stimulation for long periods in balanced salt solutions (Na + K + Ca + Mg) and in solutions lacking Ca (Na + K + Mg). Other experiments are shown in which different anions were tested. The results indicate that luminescence and irritability continue much longer in solutions of Na_2SO_4 and $MgSO_4$ than in NaCl and $MgCl_2$. These various results are rather puzzling but they show that the effects of different ions on the luminescent response are bound to be very complex. The persistence of luminescence and irritability in solutions of $MgCl_2$ and $MgSO_4$ for 3–4 hr. is extraordinary, in view of the general anaesthetic action of these salts on marine invertebrates. It is still uncertain whether squid photophores are subject to direct control, and these results may reveal only duration of vitality.

DISCUSSION AND CONCLUSIONS

It appears to be firmly established from this and earlier studies that the luminescent responses of polynoids are under nervous control. The nerve fibres supplying the photocytes have been revealed by histological means, and their activity demonstrated by experimental methods. These nerve fibres are excitatory nerve fibres, and appear to be the only ones supplying the photocytes (Bonhomme, 1942; Nicol, 1953).

In the normal intact animal the chain of neural events leading to the appearance of luminous flashes appears to involve: excitation of peripheral receptors or sensory endings, which abound on the dorsal surface of the elytra, and are probably distributed over the general surface of the body as well; initiation of impulses in afferent fibres running into the nerve cord; reflex transmission of impulses in efferent fibres to a peripheral ganglion lying in the elytrum; relaying of nervous impulses into terminal efferent fibres which radiate outwards from the elytral ganglion to the photocytes (Pflugfelder, 1933; Bonhomme, 1942).

Some of the evidence for this reflex arc is indirect, but there seems little reason to doubt that it represents one method by which the luminescent response is evoked. When any localized region of the body is stimulated mechanically, luminescence appears not only in the elytra of the region directly stimulated, but in more distant segments as well. Excitation is thus initiated by peripheral stimulation, and is transmitted longitudinally along the nerve cord. Electrical stimulation of the nerve cord also evokes normal luminescent flashes.

Turning now to the efferent pathways, we find that the normal response of a scale is a long series of discrete rhythmic flashes. Evidence has been presented that flashing is controlled by a ganglion situated in the centre of the elytrum. In an entire scale, or portion of a scale containing the ganglion, a single shock sets the scale flashing, as the result of rhythmic discharge from the ganglion. It may well be that, in the intact animal, one or a few impulses are sent to the elytral ganglion from the nerve cord, and initiate rhythmic discharge and flashing. Of this we have no knowledge as yet.

A second method of stimulation, operating under natural conditions, is traumatic injury. Transection of the body evokes flashing posterior to the cut, but not anterior. I have previously hypothesized the existence of synaptic resistance in longitudinal pathways through the nerve cord to explain this phenomenon. Owing to the sensitivity of these animals and the ease with which luminosity is exhausted, it may prove difficult to get information on this problem.

Autotomy or removal of a scale also starts it flashing. Excitation follows from injury potentials established when the nerves are severed. Persistent discharge in nerves after injury is not unknown in other animals. It forms the basis of Parker's hypothesis concerning caudal bands in fish (Parker, 1948). Adrian (1930) has demonstrated the occurrence of a persistent discharge of impulses in mammalian nerves after injury. The persistent discharges arise from the injured ends of the nerve fibres, the permanent depolarization of the injured region acting as a stimulus to the intact part of the fibre. The frequency of discharge is high, and the time relations indicate that the nerve fibres are responding to an excitation which outlasts the refractory period of the fibre; the refractory period thus determines the rate of discharge. There are several reasons for believing that the same process is not operating in autotomized polynoid elytra. First, rhythmical flashes (following electrical stimulation) can be induced only in a preparation containing a ganglion; bits of scale lacking a ganglion respond by a single flash to each stimulus. The ganglion, accordingly, is necessary for luminescence. Secondly, the rate of flashing is relatively slow, 10 to 1 per sec., far below the rate which would be determined by the refractory period of nerve, if this were the controlling factor.

It seems that a stimulus, be it injurious, mechanical, nervous, or electrical, excites the pre-ganglionic nerve fibres[1] and the nerve cells. A prolonged excitatory state is set up in the elytral ganglion, which far outlasts the duration of a single impulse, and is responsible for the initiation of impulses in the efferent fibres. These events are reflected in the luminous flashes which appear rhythmically for some time after stimulation. It appears as if the pre-ganglionic stimulus, on exciting the ganglionic cells, sets up a rhythmic oscillatory state in the latter, with a rather slow period of discharge, beginning at about 0·1 sec. and increasing to 1 sec. Presumably at the peaks of the cycle, excitation rises to a sufficient level to fire the post-ganglionic fibres, and send off impulses to the photocytes.

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[1] There is no clear-cut evidence, as yet, for the existence of synapses in the elytral ganglion. T-shaped neurones, for example, could be involved.

A search of the literature reveals no strictly comparable system, although analogies exist. Rhythmic ganglionic discharges of an inherent nature have been recorded from the brain and nerve cord of different invertebrates (Bullock, 1947), and occur in certain isolated peripheral ganglia, e.g. cardiac ganglion of *Limulus* (Armstrong, Maxfield, Prosser & Schoepfle, 1939; Bullock, Burr & Nims, 1943). A constant background of spontaneous discharge is characteristic of certain peripheral receptors, e.g. of vestibulo-lateralis system of fishes, and the frequency of such discharge may be rather low, around 5 impulses/sec. (Suckling & Suckling, 1950; Lowenstein & Roberts, 1950). Caudal photoreceptors have been identified in the nerve cord of the crayfish *Cambarus*, which discharge at a high frequency when illuminated. Of particular interest is the observation that after illumination ceases, an after-discharge persists for several seconds, during which the impulses decline to a spontaneous level (Prosser, 1934). Recordings from the isolated visceral ganglion of *Aplysia* have revealed the existence of spontaneous rhythmic oscillations of potential, arising in nerve cell bodies. These oscillations, followed by discharges in efferent nerves, show rather long periods, about 420 msec. (minima, 70 msec.) (Arvanitaki & Cardot, 1941).

As the above résumé reveals, other instances are known in which rhythmic oscillations of potential, sometimes evoked by external stimulation, lead to nervous discharges, and the frequencies are often within the range encountered in polynoid flashing (1–15/sec.). By postulating the occurrence in ganglionic neurones of rhythmic oscillations of potential corresponding to the rhythmic flashes, one can suggest explanations for certain characteristics of the rhythmic process. We have seen that long pauses, several seconds in duration and far exceeding the length of a normal period, sometimes intervene between successive trains of flashes (e.g. Fig. 8c). It is not unlikely that these quiet hiatuses indicate periods of subliminal potential oscillations, below the threshold for firing efferent fibres. Owing to the fact that the luminescent flashes show progressive fatigue leading to extinction, it is difficult to secure much information about the maximal duration of the underlying excitatory process responsible for the rhythmic flashes; certain favourable preparations suggest a maximal duration of 1–2 min. at least for supraliminal activity.

Curves showing variations in flash frequency with time and succession have been presented in a previous paper (Nicol, 1953). The frequency is high at first, rapidly falls off to a steady level of approximately 1/sec., and after a variable period, often about 1 min., decreases to extinction. Now if the time relations of this rhythmic flashing are a true index of underlying potential oscillations, then we can conclude that an external stimulus or nervous impulse excites the nerve cell bodies, and initiates rhythmical oscillations and discharge. At first high, the frequency of oscillation quickly falls off during the first few seconds. It has also been discovered that with repeated stimulation, the number of rhythmic flashes which can be induced by a single stimulus declines until finally only a single flash is induced by one stimulus. In the latter event rhythmic flashing can be evoked again by delivering a burst of high frequency pulses. It appears as if the excitatory state of the neurones is itself subject to fatigue. After repeated periods of stimulation the number of rhythmic oscillations falls off as well, until only a single discharge is evoked. These speculative remarks indicate the need for electronic recording of action potentials.

Peripheral ganglia, associated with the appendages, have been described in other errant polychaetes. In *Nereis* (Nereidae) and *Hermione* (Aphroditidae), there are parapodial ganglia at the bases of the parapodia, and in the latter animal these connect with cirrus ganglia near the cirri. Sensory nerves and motor nerves to parapodial muscles pass to and from these centres (Schneider, 1902; Hanström, 1928). According to Maxwell (1897) the parapodial ganglia of *Nereis* act as local reflex centres for parapodial movements. It is possible that the elytral ganglia of polynoids are derived from parapodial or cirrus ganglia, such as those existing in *Hermione*.

The increase in height of subsequent luminescent responses indicates the existence of some facilitatory phenomenon. The progressive increment in intensity of consecutive responses is analogous to staircase in the vertebrate heart, or tension-rise in crustacean muscle. The prolonged facilitatory period, up to 4 min., would appear to preclude the operation of some persistent electrical potential. Neither is there any indication that chemical transmitters—acetylcholine and adrenaline—are involved in the luminescent response. There is some evidence for the existence of cholinergic nerve fibres among annelids, however, with the distinct possibility that acetylcholine is concerned in neuromyal transmission (evidence reviewed by Prosser, 1950). Evidence also exists for sensitivity to adrenaline in a few species (earthworm gut, vascular system of the leech) (Gaskell, 1914; Hanström, 1939; Ambache, Dixon & Wright, 1945). The ineffectiveness of atropine and curare on the luminescent response offers no conclusive evidence, since these two drugs fail to block neuromyal transmission in some other annelids (Nicol, 1952*d*).

It appears, then, that either nervous excitation of the luminescent gland cells is radically dissimilar from neuromyal transmission, or that the neuroglandular junction is inaccessible to the reagents used. Nachmansohn (1950) has developed a hypothesis of this kind to explain differences between the effectiveness of methylated quaternary ammonium salts and tertiary amines, e.g. eserine. It may be noted that Bonhomme (1942) has asserted that the nerve fibres supplying the photocytes of polynoid worms may penetrate into the glandular cells. In this event a post-synaptic membrane, comparable to that between nerve and muscle, would not exist, and the drugs would have no extracellular interface upon which to act. The existence of distinct microscopic granules of luminescent material in the cytoplasm of the photocytes must mean that these cellular inclusions are provided with distinct intracellular interfaces. It is tempting to regard these interfaces as the site of control of the luminescent flash, by ionic exchange, release of energy-bearing substance, or other mechanism. Facilitation, then, may represent changes at these loci. The influence of the ionic environment, particularly of changes in sodium and potassium, is described on pp. 243–6. ATP, which has a positive effect on the luminescent reaction of certain animals, fails to revive luminescence in extracts of polynoid scales after the initial glow has died away (see Appendix) (Harvey & Haneda, 1951).

Luminescent responses, consisting of brief flashes, are recorded for other marine and terrestrial animals, and it is of interest to discover whether the same or similar regulating mechanisms may be operative in these animals. Certain calyptoblastic hydroids give off intermittent repeated flashes when mechanically stimulated, possibly representing repetitive discharge in the nerve net. Intermittent flashes are reported in nudibranch molluscs (*Kaloplocamus*, *Plocamopterus*), a pulmonate (*Dyakia*), euphausiids (*Nyctiphanes*), and decapod crustacea (*Sergestes*). The regulating mechanism in these animals has not been investigated. In cephalopods and fishes which give off discrete flashes the photophores are either innervated and subject to direct nervous control (myctophids or lantern-fish), or periodically uncovered by screening devices (fire-fly squid *Watasenia*; stomiatoid teleost *Astronesthes*). Rhythmic luminous responses (flashing) have been most studied in fire-flies (Lampyridae). In the majority of luminescent species the response takes the form of an intermittent glow, pulsation, or series of flashes. In *Photuris pennsylvania*, for example, males flash at a rate of about 3/sec., and each flash normally appears as a symmetrical response, lasting around 0·15 sec., and rising to a peak in half that time. There is still some uncertainty how these flashes are controlled, but much suggestive evidence exists that flashing is regulated by provision of oxygen through tracheal end-cells (see Buck, 1948, and Harvey, 1952, for review of literature). In Fig. 10 some flash-curves are presented of different species under various conditions. Luminescent flashes appear to develop a little faster in polynoids than in *Photuris*, and to be of slightly shorter duration. An apparent difference between the flash-curves of the two animals lies in the symmetrical appearance of the curve for *Photuris*, and the slower progress of decay in the curve for *Acholoë*.

The symmetrical appearance of the response of *Photuris* (likened to a distribution curve) probably depends upon summation of the flashes of a great many sub-units, all slightly asynchronous, while the photocytes in each scale of *Acholoë* flash more synchronously. The decay portion of the curve (exponential) in the latter animal, therefore, more nearly represents the course of decay of the luminescent reaction in each photocyte. Added to Fig. 10 are two curves for oxidation of *Cypridina* luciferin and luciferase, mixed prior to admission, and in the presence, of oxygen. In this animal, at least, interaction between luminescent substrate and catalyst is a more limiting factor than oxygen availability. In intracellular luminescence (Polynoidae), the rise of intensity must represent the time course of provision of reactant(s), and interaction of reacting materials (Snell, 1932; Chance, Harvey, Johnson & Millikan, 1940).

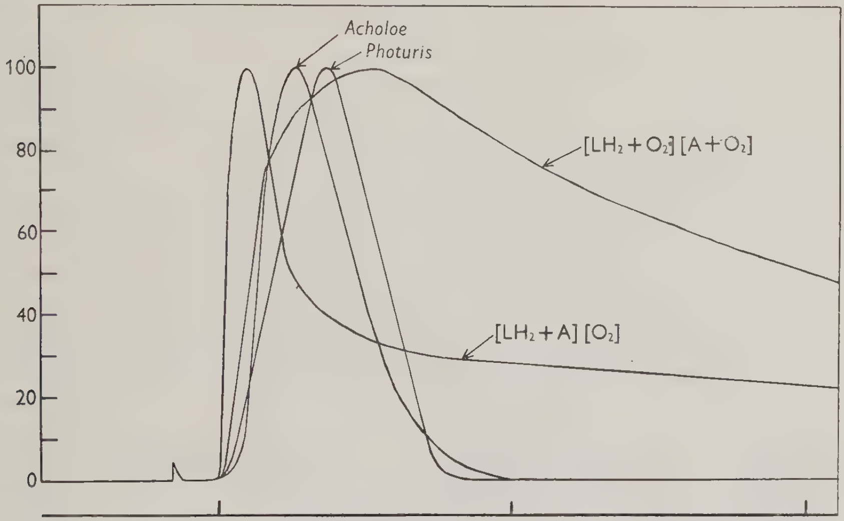

Fig. 10. Curves of luminescent responses and luminescent reactions from various sources. Luminescent responses of *Acholoë* (polynoid) and *Photuris* (fire-fly). The small pip at the left on the base line is the stimulus mark for *Acholoë* only. Two curves are shown for oxidation of *Cypridina* luciferin. Luciferin and luciferase mixed in presence of O_2 ($[LH_2+O_2]$ $[A+O_2]$), and a mixture of luciferin and luciferase to which O_2 is admitted ($[LH_2+A]$ $[O_2]$). Time scale below, intervals of 100 msec.

The luminescent responses of single scales of polynoids are usually simple flashes resulting from synchronous activity of all the photocytes, although a few records give evidence of asynchronous flashing of several units. From this it appears that a single neurone innervates all the photocytes (one neuro-effector unit) or, if more than one neurone is involved, that the several neurones show synchronous activity and fire in pace with each other. Asynchronous activity, occasionally recorded, confirms histological evidence that several neurones exist in the elytral ganglion. Synchronous activity possibly depends on interaction of cell potentials, such as takes place between contiguous nerves (*Carcinus*) or adjacent nerve cells (*Aplysia*) (Katz & Schmitt, 1940; Arvanitaki, 1942).

Part of the expenses incurred in this research was defrayed by a grant-in-aid of scientific investigations from the Royal Society.

SUMMARY

This paper describes physiological studies on the luminescent responses of several polynoids, especially *Acholoë* and *Polynoë*. Luminescent flashes have been recorded by photoelectric means, and the effects of various chemical agents explored. The results obtained have been related to, and integrated with, analogous studies on other animals, as far as possible. The principal results and conclusions are as follows.

(i) The normal luminescent response of a scale is a series of flashes, which can be evoked by a single electrical shock and is controlled by the nervous system. A very strong shock produces a prolonged glow, exhausting the scale, and resulting from direct excitation of the glandular cells.

(ii) Consecutive flashes, following each other at suitable intervals, show an increase in intensity. This is in part due to summation when the frequency is high enough to permit fusion, but is to a great extent attributable to facilitation. A curve for decay of facilitation is presented, and the effect is shown to last for as long as 4 min. Facilitation occurs peripherally at the neuroglandular junction or in the photocytes.

(iii) At high frequencies, above 25/sec., the flashes begin to fuse. A consequence of high-frequency stimulation is that the intensity of response is reduced, due to failure of transmission or to the existence of relative refractoriness peripherally.

(iv) Absolute refractory period (*Polynoë*) of the response lies between 9 and 16 msec.

(v) Rhythmic flashing is controlled by a peripheral ganglion in the elytrum, from which nerves radiate to the photocytes. Preparations lacking this ganglion give only a single flash to each stimulus.

(vi) At the beginning of a response the flash interval is small, around 100 msec.; this increases to 1 sec. during the course of a response; in some preparations long intervals, up to 15 sec. between successive flashes, have been noted. When preparations are subjected to consecutive periods of stimulation, the number of flashes evoked gradually falls off, suggesting fatigue of the neural mechanism regulating flashing.

(vii) Of a series of drugs tried, viz. adrenaline, acetylcholine, curarine, eserine, atropine, nicotine, and strychnine, all were without effect on flashing except the last, which blocked electrical excitation.

(viii) Isosmotic solutions of single salts and combinations of salts had the following effects: potassium caused a prolonged glow; sodium evoked rhythmical flashing; calcium increased excitability; choline chloride was without effect; magnesium acted as an anaesthetic; calcium and magnesium antagonized the excitatory effect of sodium. Potassium is thought to act directly on the photocytes, sodium on the nerves at first, and then on the photocytes.

(ix) ATP is shown to be without effect on extracts of the luminous tissues of polynoids, *Pholas* and *Polycirrus* (Appendix A).

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APPENDIX

EFFECT OF ADENOSINE TRIPHOSPHATE ON THE LUMINESCENCE OF CERTAIN MARINE ORGANISMS

Following the experiments of Harvey & Haneda (1951), I have tested the effects of adenosine triphosphate on luminescent extracts of several marine animals, viz. *Pholas dactylus*, *Polycirrus caliendrum*, *Acholoë astericola* and *Polynoë scolopendrina*. The luminescent tissues of these animals were ground up with sand in a little sea water (10 ml.), until the light disappeared. A solution of 5 mg. ATP was then added to this extract, or to the supernatant fluid after centrifugation. In none of these preparations did ATP revive luminescence.

I have to thank Dr G. Y. Kennedy of the Cancer Research Laboratory, University of Sheffield, for a sample of the barium salt of ATP. This was converted to the sodium salt by the procedure described in Carter (1942).

J. Mar. biol. Ass. U.K. (1954) 33, 257–264
Printed in Great Britain

A SPRING-LOADED BOTTOM-SAMPLER

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(Text-figs. 1–3)

INTRODUCTION

For routine sampling of the infauna of the sea bottom an apparatus is required which is simple, easily handled, and which can give samples of consistent volume from different types of bottom and in varying weather conditions.

The Petersen sampler and its modification, the van Veen, are simple and easily handled and give reasonably consistent samples from some types of soil. But on hard ground they are less satisfactory, and in rough weather they tend either to land unevenly on the bottom, thereby taking smaller samples of variable volume, or to release in mid-water with the rolling of the ship, so that no sample is taken.

A new sampler, which is in use at the Plymouth Laboratory, has been described by Holme (1949). We are glad to be able to acknowledge here the kindness of the Director and Mr Holme in arranging for us the early supply of one of these samplers. However, in spite of its improvements, it has certain disadvantages which are well summed up in the author's own words 'the area sampled ($\frac{1}{20}$ m^2.) is, however, rather small, and the apparatus rather heavy, and so difficult to work except in calm weather'. In using Holme's sampler from Aberdeen this last point, the difficulty of using it in anything but calm weather, was found to be its greatest disadvantage.

When bottom-fauna work has to be fitted into a wider programme, it is often impossible to wait for suitable conditions, and the need was still felt for a sampler which could be used with confidence in any weather. The apparatus described here was designed and constructed, by the first author, in an effort to produce a sampler which would give consistent results even in bad weather and on 'difficult' grounds. It was constructed at the Northern Engineering Works, Peterhead, and we wish to express our thanks to the proprietors for their interest and assistance.

THE SAMPLER

The apparatus is shown in Fig. 1. It consists essentially of a spring-loaded bucket carried in a frame. In use, when the apparatus comes to rest squarely on the bottom, the springs are released and drive the bucket into the soil; hauling on the warp then closes the sampler.

The bucket, which is semicircular in cross-section, is hinged on an axle along the centre line, and is opened and closed by means of two arms (Fig. 3 A).

It is carried by an inverted U-shaped bridle which is attached at both ends of the axle. The horizontal bar of the bridle is bored so that the bridle and bucket can move vertically on a tubular guide (Fig. 1) fixed at the centre of the frame. This vertical movement is limited to 3 in. by stops. Two springs

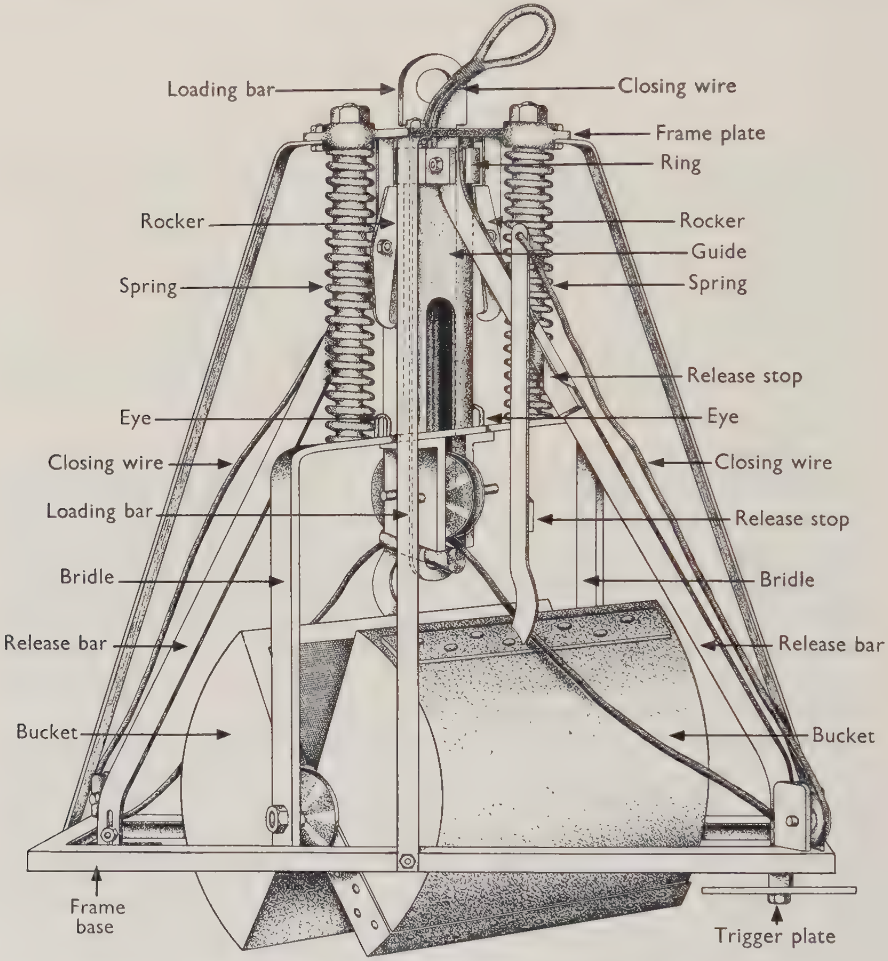

Loading bar
Closing wire
Frame plate
Ring
Rocker
Rocker
Guide
Spring
Spring
Release stop
Eye
Eye
Closing wire
Closing wire
Loading bar
Release stop
Bridle
Bridle
Release bar
Release bar
Bucket
Bucket
Frame base
Trigger plate

Fig. 1. Diagram of sampler, in the unloaded position (overall dimensions, 26 × 26 × 20 in.).

(each loading 50 lb./in.) are placed so that their lower ends bear against the bridle and their upper ends against the underside of the frame plate. Each spring is located by an internal rod on the bridle (Fig. 3A) which slides into a tube attached to the frame plate. To the middle of the bridle is secured a loading bar which passes up the centre of the guide and projects through the frame plate. In loading, this bar is pulled up by means of a loading lever, and the bucket is raised against the pressure of the springs until two eyes on the bridle come within reach of two rockers attached to the frame plate. The rockers engage in the eyes and hold the bucket in the loaded position against the pressure of the springs. The faces of the rockers bearing in the eyes are set at such an angle that a horizontal component of the pressure tends to free the rockers from the eyes, releasing the bucket. This movement of the rockers is prevented by a ring against which the upper ends of the rockers bear when the apparatus is in the loaded position. The ring is fixed by knuckle joints to two release bars which terminate in trigger plates below the frame at diagonally opposite corners. When the sampler strikes the bottom squarely, the pressure on both trigger plates causes the ring to move up. This allows the rockers to swing free of the eyes and the open bucket is forced into the soil by the springs. When the instrument is set the loading bar may be removed (as in Fig. 2), but when the sampler is in continuous use it has been found convenient to leave the bar in position.

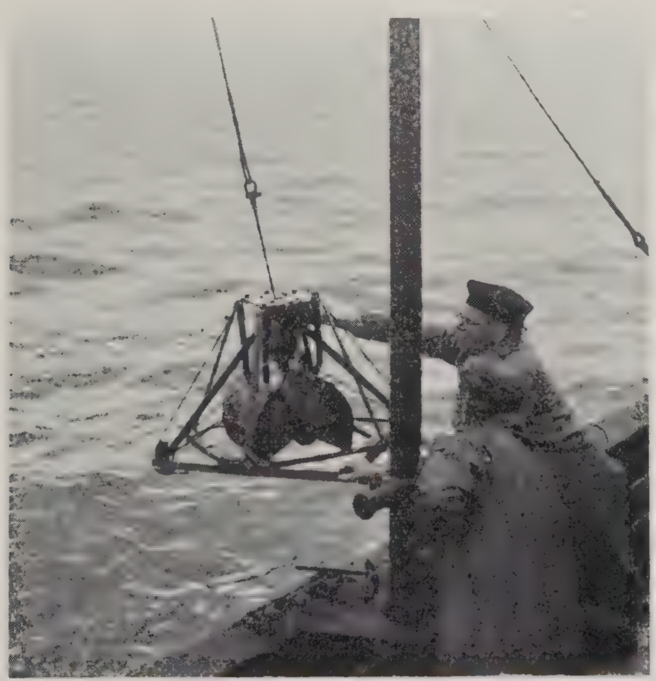
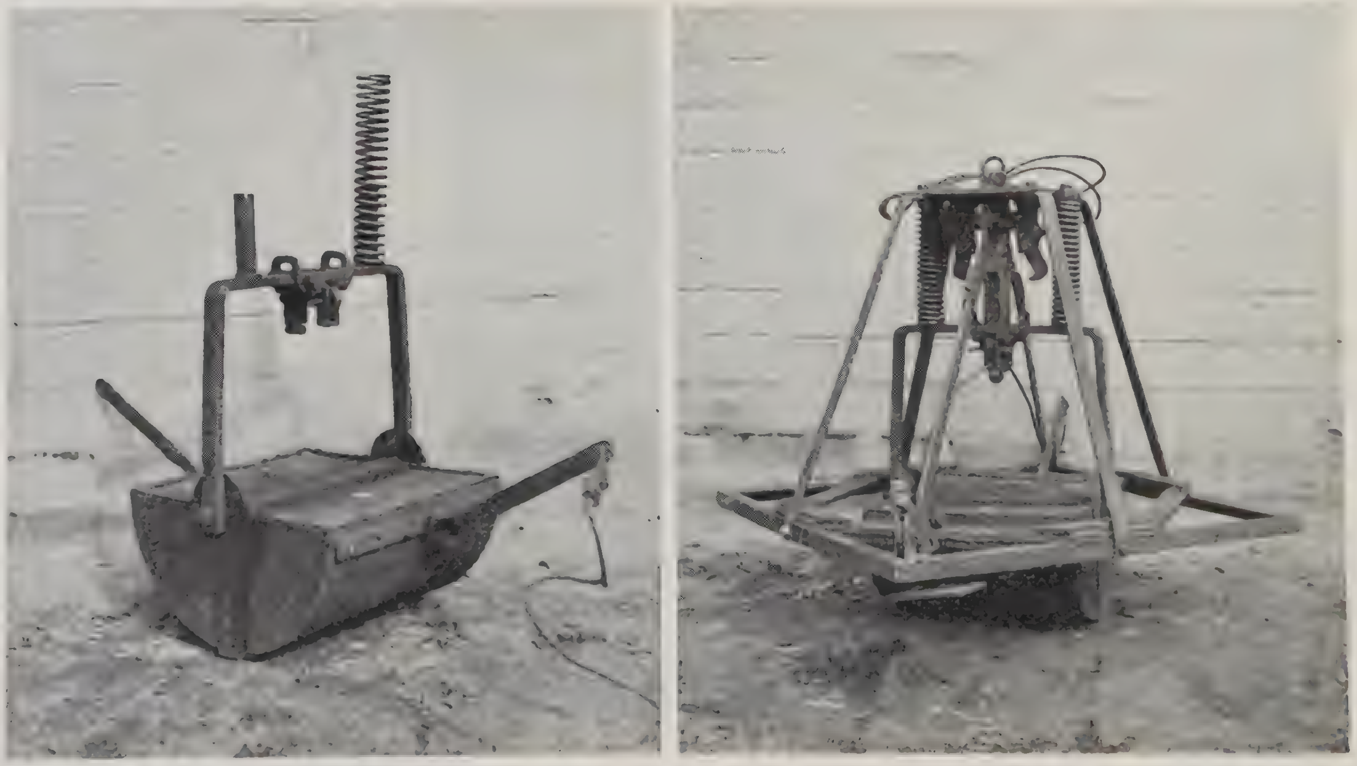


Fig. 2. The sampler in use at sea. The instrument is set and is about to be lowered. The loading bar has been removed.

A wire attached to the end of each arm of the bucket passes over pulleys on the frame and bridle, up through the centre of the guide, and is shackled above the frame plate to the warp from the ship. When the sampler is being lowered the closing wire bears the weight, and the bucket is prevented from closing by



A

B

Fig. 3. A, the bucket, closed. B, the sampler, closed, with the loading bar in position.

stops on the arms bearing against similar stops on the release bars (Fig. 1). The release of the bucket when the sampler hits the bottom disengages the arms from the stops so that hauling on the warp first closes the bucket, then lifts the sampler off the bottom.

The bucket has a radius of $17\frac{1}{2}$ cm. and a length of 32 cm., and is constructed of $\frac{1}{8}$ in. (3·2 mm.) sheet metal. The area of bottom sampled is $\frac{1}{10}$ m.^{2}. The top of the bucket is covered with fine-mesh gauze. The arms which are riveted to the bucket are 28 cm. long and are of $1 \times \frac{1}{4}$ in. flat iron. The bridle is made from two pieces of $1\frac{1}{4} \times \frac{1}{2}$ in. flat iron bent at right angles, and welded to a $\frac{1}{2}$ in. plate 3 in. square which is bored to slide on the central guide. The guide is 10 in. long and of $1\frac{3}{4}$ in. diameter tube. It has a $\frac{3}{4}$ in. slot on each side extending upwards from the lower end for 5 in. The base of the frame consists of four bars of $1 \times \frac{1}{8}$ in. angle iron 26 in. long, and from each side of the frame an upright bar of $1 \times \frac{1}{4}$ in. flat iron runs to the frame plate, which is 8 in. square and $\frac{1}{4}$ in. thick. The angle iron used for the base proved to be too light, and was strengthened by cross-bars (see Figs. 2 and 3B). The loading bar is $1 \times \frac{1}{4}$ in. flat iron and has a loop welded to the top. The apparatus is 20 in. high and weighs 100 lb. (45 kg.).

RESULTS

Previous work (Thamdrup, 1938) indicates that the van Veen is more efficient than the Petersen grab, and experience at Aberdeen with the van Veen, Petersen, and Plymouth samplers has shown that the van Veen was the most satisfactory over a wide range of conditions. The van Veen has thus been in general use at Aberdeen, and since it is obvious that any new sampler must be compared with the most efficient of the older models, tests have been carried out using the $\frac{1}{10}$ m^2. van Veen and the Smith sampler.

The samplers were used from the ship at anchor on various grounds and at different depths, the order of use being determined from a table of random numbers. The volumes, in litres, of material taken in each sample are set out below:

(1) Dornoch Firth, 9 m. depth, muddy sand

Smith sampler	3·75	3·5	2·5	3·25	Mean 3·25 per $\frac{1}{10}$ m.^{2}
v. Veen sampler	4·0	3·5	3·0	2·5	Mean 3·25

(2) Dornoch Firth, 13 m. depth, muddy sand

Smith sampler	4·5	2·5	4·5	4·5	Mean 4·0 per $\frac{1}{10}$ m.^{2}
v. Veen sampler	2·25	2·5	2·25	2·0	Mean 2·25

(3) Dornoch Firth, 24 m. depth, muddy gravel

Smith sampler	4·75	4·75	5·0	4·0	Mean 4·6 per $\frac{1}{10}$ m.^{2}
v. Veen sampler	3·25	4·0	3·0	2·75	Mean 3·25

(4) Dornoch Firth, 24 m. depth, mud

Smith sampler	5·0	4·0	4·75	5·5	Mean 4·8 per $\frac{1}{10}$ m.^{2}
v. Veen sampler	3·0	3·0	3·5	3·25	Mean 3·2

(5) Aberdeen Bay, 37 m. depth, hard sand

Smith sampler	3·5	1·5	4·0	4·0	3·5	3·5	3·0	Mean 3·3 per $\frac{1}{10}$ m.^{2}
v. Veen sampler	2·0	3·5	2·5	2·0	1·5	0·5	3·5	Mean 2·2

For test number (5) above, a detailed analysis was made and the frequencies of the various organisms taken are shown in Table I. In nearly every comparison the Smith sampler gave a higher population estimate, and statistical analysis showed that the difference between the two samplers in this respect was significant at the 1% level.

TABLE I

Aberdeen Bay. Depth 37 m. Bottom, hard sand. The mean numbers of animals taken in seven dips with the van Veen and Smith samplers respectively.

	van Veen sampler	Smith sampler
Gastropods	0·1	2·0
Nucula	20·4	23·0
Thyasira	1·1	1·3
Montacuta	2·7	1·6
Cyprina	1·3	2·3
Dosinia	11·1	13·6
Venus fasciata	2·3	3·9
Tellina fabula	5·6	6·1
Abra	3·9	6·1
Gari fervensis	1·3	1·1
Cultellus	0	0·4
Thracia	1·6	2·3
Other lamellibranchs	0·3	0·7
Ophiuroids	3·0	4·4
Echinoids	3·9	4·0
Crustacea	3·4	6·0
Aphroditidae	2·0	2·4
Goniada	2·9	3·3
Nepthys	1·7	2·6
Lumbriconereis	1·3	1·6
Other errant polychaetes	0·3	1·1
Ariciidae	2·9	5·1
Spionidae	0·7	2·0
Magelona	0·3	1·0
Cirratulidae	1·7	2·0
Chlorhaemidae	1·3	1·6
Other sedentary polychaetes	0·7	0·9

On two occasions the new sampler was compared with a $\frac{1}{5}$ m.^{2} Petersen grab, with the following results (volumes in litres):

(1) Smith Bank, 37 m. depth, fine sand

Smith sampler	4·0	6·0	5·0	4·0	4·0	4·0	3·5	4·0	5·0	Mean 4·4 per $\frac{1}{10}$ m.^{2}
$\frac{1}{5}$ m.^{2} Petersen sampler	3·0	4·0	5·0							Mean 4·0 per $\frac{1}{5}$ m.^{2}

(2) Off Smith Bank to northwest, 73 m. depth, mud

Smith sampler	2·5	2·0	4·0	5·0	2·5	2·0	Mean 3·0 per $\frac{1}{10}$ m.^{2}
$\frac{1}{5}$ m.^{2} Petersen sampler	2·5	3·0					Mean 2·75 per $\frac{1}{5}$ m.^{2}

It is of interest that the smaller sampler tended to take a larger volume.

Apart from such specific tests, an indication of the relative performance of the two samplers is given by figures collected during routine surveys over a period of several months. The following comparisons are from recent data (volumes in litres):

(1) Smith Bank, 37–40 m. depth, fine sand			
Smith sampler	52 dips	Mean volume 3·9	Range 1·0–7·0.
v. Veen sampler	165 dips	Mean volume 3·4	Range 0·25–6·0.
(2) Smith Bank, 37–40 m. depth, shell gravel			
Smith sampler	9 dips	Mean volume 5·0	Range 4·0–7·0.
v. Veen sampler	15 dips	Mean volume 4·5	Range 0·5–6·0.
(3) Off Smith Bank to northwest, 62–73 m. depth, mud			
Smith sampler	9 dips	Mean volume 3·0	Range 2·0–5·0
v. Veen sampler	9 dips	Mean volume 2·1	Range 0·5–3·5.

Discussion

The above tests show that the new sampler is more efficient than the van Veen in the areas visited. Perhaps the most significant feature, however, is not shown by these data. The opportunity to compare the two instruments usually arose during a general bottom-sampling programme. On several occasions the comparison had to be abandoned because bad weather prevented the van Veen from operating. On each occasion, however, it was found possible to complete the programme with the Smith sampler only, which continued to give samples of reasonably consistent volume even under the most trying weather conditions.

The success of the new sampler in bad weather is due to its inability to release until resting squarely on the bottom. If it strikes the bottom unevenly only one of the release arms is operated and, since the arms are connected to the ring by knuckle joints, movement of one arm merely causes the ring to pivot. Release cannot take place until the other arm is also raised, i.e. until the apparatus is settled on the bottom.

A distinctive feature of the new sampler is connected with its method of closing. A great part of the superiority of the van Veen over the Petersen grab is probably due to the leverage effect exerted by the arms in closing the jaws. This leverage effect is greatest when the van Veen is fully open, and decreases as the jaws close and the arms come together. In the new sampler, however, the pull on the arms is downward, and the leverage effect increases as the sampler digs into the ground and reaches a maximum as the jaws come together.

Another feature of the new sampler is the gauze covering on top of the bucket. Because of this the down-wave produced on descent is relatively small, and this may help to account for the high proportion of active surface-dwelling forms, such as euphausiids and amphipods which have been found in the new sampler. In surveys of Smith Bank, for example, the new sampler took on the average twice as many amphipods as the van Veen. Samplers with a solid upper surface must set up a considerable down-wave, and this will tend to wash small surface animals out of the sampler's reach. It is also important that, since the bucket closes completely, no material is lost in hauling. Further, the mesh of the gauze covering is considerably finer than that of the gauze used in sieving.

In its present form, the theoretical maximum depth to which the new sampler can bite is 7 cm., and it is of interest to note that the depth to which it has in fact been digging, calculated from its average volume of 4 l., is 6·7 cm. It is felt that there is a considerable reserve of power in the sampler, and alterations are being contemplated which would give an increased depth of bite.

Once the routine of loading and operating the new sampler has become familiar, it can be used almost as quickly as the van Veen. Working from F.R.S. *Explorer* at a depth of 40 m. the average time taken to complete three dips with the van Veen was 14 min., and with the new sampler 15 min.,—a negligible difference.

SUMMARY

A new bottom-sampler is described. It covers a surface area of $\frac{1}{10}$ m.^{2} and digs to a depth of almost 7 cm. The sampler consists of a bucket which is driven into the soil by springs and is closed by the hauling in of the warp. The apparatus can sample only when resting squarely on the bottom, and was designed to work on hard sand and in bad weather. It has been shown to be successful in both these respects.

Specific tests and experience under various conditions show it to be more generally satisfactory than any of the other samplers used.

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J. Mar. biol. Ass. U.K. (1954) 33, 265–290
Printed in Great Britain

STUDIES ON THE CULTURE OF A MARINE DIATOM

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(Text-figs. 1–16)

Little is known of the biochemistry of diatoms, although many workers have reported growth experiments with the unicellular algae of the marine phytoplankton. Experiments have often been performed without due regard for the appropriate control of physical and chemical conditions. Many reports contain only incomplete data of the growth under a given set of conditions, and it is often impossible to say whether the effects recorded are upon the growth rate, the total crop, or both. Other studies have been reported which included the addition of organic matter to cultures which were only uni-algal and not bacteria-free. At the present time even the mere maintenance of stock cultures of the marine unicellular algae is perforce an empirical matter. Results in replicate cultures often show gross differences in growth that are apparent on inspection by eye alone, and insufficient information is available regarding the nature of these variations in growth to allow the rational development of improved culture media. Due therefore to a lack of suitable techniques, most of the results available are difficult, if not impossible, to interpret in terms of the biochemical activities of the algae.

Quantitative measurements of the growth of bacterial cultures have been widely used by Hinshelwood, Monod and others (Hinshelwood, 1946; Monod, 1942). The technique may be limited in its application, for example in the elucidation of metabolic pathways by the permeability of the cell wall to intermediates, but such studies can furnish much valuable information. Investigations have shown that the growth of bacteria as measured by an increase in cell numbers or cell material generally obeys certain simple rules. The growth can thus be defined by a number of growth constants which reflect the physiological behaviour of the cells. Similar considerations should apply to the growth of any unicellular organism. Comparison of the growth constants under various conditions should therefore provide some information about the suitability of these conditions for culture work, allow the rational development of improved culture media, and make possible fuller interpretation of the results of growth experiments.

It is normal practice at the moment to use natural sea water as a base for culture medium for the marine unicellular algae. This is usually enriched with further amounts of phosphate, nitrate and soil extract. The preliminary investigation reported in this paper has been restricted to experiments in medium of this type.

DEFINITION OF TERMS USED

In this paper the terminology used by the previous workers on the kinetics of bacterial growth has been largely adopted. The nature of some of the growth phases obtained, however, is such as to lead to difficulties if the traditional nomenclature is followed. These exceptions will be discussed later as they arise. The various growth phases are best described in terms of an idealized growth, as shown in Fig. 1. It is obvious that the growth may be expressed in terms of either the increase in cell numbers (cell concentration) or the increase of cellular material (cell density). Since the technique used for estimating growth measures cell concentration, in all that follows the term growth will be used to mean an increase in cell numbers per unit volume.

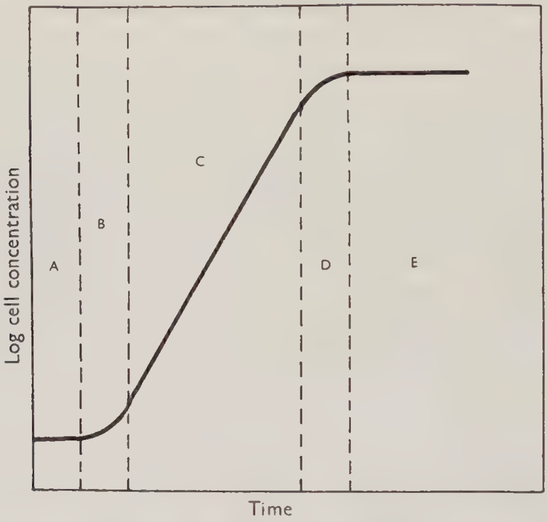


Fig. 1. An idealized growth curve showing the five phases of growth. A, initial stationary phase; B, phase of increasing growth rate; C, exponential phase; D, phase of decreasing growth rate; E, final stationary phase.

When cells of the test organism are inoculated into suitable culture medium cell division may or may not start at once. In the latter case there is usually an initial stationary period, during which no increase in cell concentration occurs, though there may be an increase in cell density. The initial stationary phase is followed by a phase of increasing growth rate when the rate of cell division increases with time up to a maximum value. The initial stationary phase and the phase of increasing growth rate are often considered together and called the 'lag phase'. The lag phase is followed by a phase of regular exponential growth, the cell concentration increasing with time in geometrical progression according to the equation:

$$n = n_0 e^{kt},$$

where n_0 is the original cell concentration and n the cell concentration at time t, k being a constant. During this exponential growth the division rate of the cells remains constant. The extent of the exponential phase is limited by the utilization of nutrients or other modifications in the culture medium caused by the growth of the organism, and it finally leads to a phase of decreasing growth rate. During this phase the growth rate decreases with time until it becomes zero. This last phase is known as the final stationary phase.

It is convenient to depict growth graphically on a plot of $\log_2 n$ (or in some cases $\log_2 n/n_0$) against time. On a plot of this sort an increase of one unit on the ordinates corresponds to one cell division. The lag time is usually measured by a plot of $\log_2 n/n_0$ and extrapolating to zero when the intercept on the time axis gives the lag time. The exponential growth may be characterized by the mean generation time or the time taken for the cell concentration to double. The total growth is the difference between the initial and the final (i.e. during the final stationary phase) cell concentration. These three constants, the lag time, the mean generation time and the total growth, largely characterize the growth obtained under a given set of cultural conditions.

METHODS

Organism Used and Maintenance of Stock Cultures

The diatom used in these studies was a bacteria-free strain of *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima* Allen & Nelson. The species was first isolated by Allen & Nelson (1910) and has since been maintained at the Plymouth Laboratory in the Miquel sea-water medium of Allen & Nelson. Unlike many other marine algae, this diatom will grow indefinitely in the absence of soil extract. Because of this, the organism was considered more suitable for the initial studies of the effect on growth of the basic physical and chemical factors than one needing the unknown factors of soil extract. A bacteria-free strain of the organism was obtained by treatment with penicillin and streptomycin as previously described (Spencer, 1952).

Stocks of the organism were maintained by periodic aseptic subculture on solid media (see later for composition). After good growth was obtained, the cultures were removed from the light and stored in the dark at 2° C. Such stored cultures show little loss of viability for periods of up to 6 weeks. Stocks were normally kept on slopes in rimless Pyrex boiling-tubes stoppered with cotton-wool and illuminated in a north window. The algal growth obtained on solid medium is sometimes somewhat mucilagenous and individual colonies may be quite hard. In neither culture is the growth conducive to even spreading by means of a wire loop. It has been found convenient to subculture the stocks by scraping selected growth off a slope with a wire loop and suspending, by agitation, in sterile sea water until the latter is just observably turbid. Loop-fulls of this suspension may then be spread evenly with a wire loop on the surface of a slope. The resulting growth then consists of numerous discrete colonies. The solid medium used will allow a good growth of many marine bacteria and its use for the maintenance of stocks is useful, since any gross bacterial contamination will at once show itself. The absence of obvious bacterial colonies is nevertheless no proof of sterility. Routine periodic checks of freedom from bacterial and other contamination were carried out by inoculation into a range of bacteriological media (Spencer, 1952) of 1 ml. samples of subcultures in liquid medium.

Passage through a subculture on solid media has a marked morphological effect on the cells. The original stock culture of the organism contained both the normal and the tri-radiate type of cells with a preponderance of the latter (Wilson, 1946). The first visible growth on solid media consisted entirely of naked cells, but normal cells appeared and soon predominated. On subculture to liquid medium, all naked cells disappeared and the culture consisted entirely of normal cells. After many serial subcultures in liquid media the tri-radiate form has not re-appeared. These observations seem in the main to confirm the findings of Barker (1935).

Culture Media

For reasons stated before, the present work has been confined to an examination of growth in media made up from natural sea water. Freshly collected off-shore water (International Hydrographical Station E. 1) was filtered through a Whatman no. 3 filter-paper and then allowed to age by storage in the dark for about 1 month. This ageing process assists uniformity between various batches of media. Difficulty was often experienced due to the formation of a precipitate as a result of autoclaving. This precipitation may perhaps be due to several factors, but experience showed that by following the procedure outlined below it could nearly always be avoided. The water was initially collected and allowed to age in Pyrex glass bottles. Medium was made up with this aged sea water diluted to 75 % with glass-distilled water to give a resultant salinity of *c.* 26 ‰. The sea water was enriched at this stage with 2·0 μg. % manganese (as manganese chloride) and the nitrogen source (normally sodium nitrate) at the required concentration. It was then dispensed into the culture vessels in use, and after plugging with cotton-wool these were sterilized by autoclaving at 5 lb. pressure for 30 min. After cooling and being allowed to re-equilibrate with the carbon dioxide of the air, the medium was further enriched by additions of 10·0 μg. % iron (as ferric citrate-citric acid: Rodhe, 1948) and phosphate at the required concentration (usually as disodium hydrogen phosphate). These additions were made aseptically from sterile stock solutions of the required strength. In the initial experiments additional silica was added at this point. Experience, however, showed that this addition was without effect on growth at the population densities used. This is not surprising since it is certain that considerable solution of silica occurs during autoclaving (F. A. J. Armstrong, private communication). In all that follows the term 'basic medium' is used to indicate 75% sea water with additions of iron and manganese.

The solid medium used for the maintenance of stock cultures consisted of the basic medium (with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus) solidified with 1·5 g.% agar and further enriched with 0·5 g. % peptone (Oxoid brand, bacteriological peptone). Some growth is obtained on solid media with nitrate nitrogen as the sole nitrogen source, but the addition of peptone causes more prolific growth. Better growth is obtained on media containing both peptone and nitrate than with either alone. Ammonium nitrogen seems to be equivalent to nitrate nitrogen for growth on solid media.

Analytical quality reagents were used throughout.

Culture Apparatus

Details of the culture apparatus used are shown in Fig. 2. For the quantitative experiments, the organism was cultured in 25 × 150 mm. rimless Pyrex boiling tubes. The cultures were aerated by means of a small-bore Pyrex tube which passed to the bottom of the culture tubes and was drawn out to a fine jet. It was found convenient for aseptic handling and for optical determinations of culture density to have the aerating tube held for its entire length against the side of the culture vessel. This was achieved by sealing about 1 in. of the top internal surface of the culture vessel to the aerating tube using 'Araldite' cement. The tubes were plugged with cotton-wool. A small guard filter containing cotton-wool was attached by rubber tubing to the aerating tube of each vessel. The open end of the guard filter containing cotton-wool was attached by rubber tubing to the aerating tube of each culture vessel. The open end of the guard filter was protected by a small inverted test-tube during autoclaving or storage before use.

The culture vessels were cleaned by prolonged scrubbing in hot soap and water followed by continual rinsing in cold tap water and finally by two rinses with hot distilled water. After much use the culture vessels often became coated with a greasy substance which was very resistant to the normal washing procedure. This could usually be removed by boiling the culture vessels in a soap-and-soda solution followed by prolonged rinsing in hot water.

The culture tubes were held in a rack and immersed in water to within 1 in.

of their tops in a glass-sided tank. This was situated in a constant temperature room without natural light. The water on the bath was rapidly circulated and thermostatically controlled at any desired temperature with an accuracy of ±0·2° C.

Compressed air was well washed by passage through two Henrichi gas-washing bottles and then filtered by passage through an aspirator completely filled with cotton-wool. This was frequently autoclaved. The sterile air was then passed into a glass manifold which ran out over the culture vessels in the constant temperature bath. The outlets of the manifold were connected by rubber tubing to the individual guard tubes on each culture vessel. The supply of compressed air was controlled by a master pin valve and a screw clip on each manifold outlet. Air was bubbled through each tube at a constant rate of about 50 ml./min.

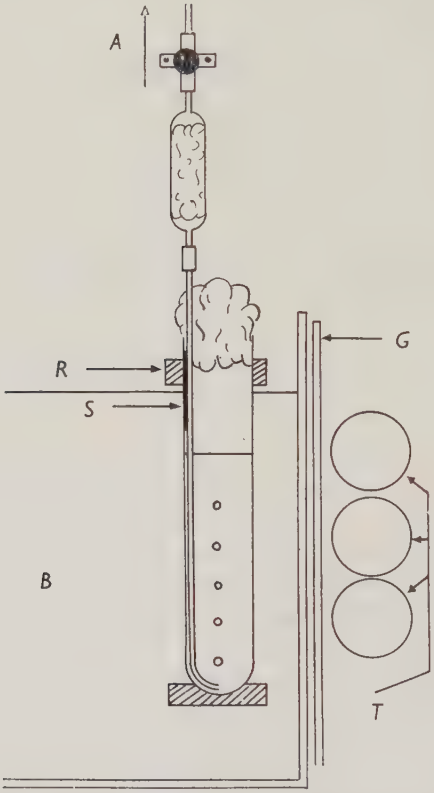


Fig. 2. Diagram of the culture apparatus used for the quantitative culture of *N. closterium*. *A*, to air supply; *B*, constant temperature bath; *G*, opal glass screen; *R*, rack; *S*, araldite seal; *T*, 80 W fluorescent tubes.

Illumination was provided by three 80 W 5 ft. fluorescent tubes. These were fixed one above the other at one side of the glass tank and extended its whole length. The intensity of illumination of the cultures could be varied by inserting opal glass screens between the lights and the side of the tank.

No attempt has been made during the present studies to investigate systematically the effects of changing the lighting conditions. Examination of the emission spectrum of 'day-light' fluorescent tubes showed them to be poor in light in the red region of the spectrum. Since chlorophyll has an absorption band in this region it was thought advisable to include some red light in the illumination. This was done by using two 80 W 'day-light' tubes and one 80 W red fluorescent tube. Preliminary experiments showed that if the culture vessels in the constant temperature bath were placed at a distance of about 3 in. from the lamps they received an incident illumination of approximately 12,000 lux. It was shown that this intensity of illumination was such that decreasing it by 25% had no effect on growth up to cell densities equivalent to a limiting initial nitrate-nitrogen concentration of 1·2 mg. %. The degree of agitation provided by aeration proved to be sufficient to prevent mutual shading by the cells at the cell concentrations used. The conditions were therefore considered to be light saturated with respect to growth.

Estimation of Growth

Several previous workers have considered the use of optical-density measurements of algal cultures as a means of estimating growth (Rodhe, 1948; Gross & Koczy, 1946; and Osterlind, 1949). For the present studies a Harvey absorptiometer (Harvey, 1948) has been modified to project a parallel beam of light through the culture. The optical density of cultures was read, using the culture vessels as cuvettes. These were inserted into the light path of the absorptiometer and held in a specially made Perspex cell containing water. This fitted into a V-shaped channel in the absorptiometer and was automatically aligned. The base of the cell contained a circular depression to hold the bottom of the culture tube, the removable lid to the cell having a circular hole of the same diameter as the culture tube. The depression and hole were made so that the culture vessel was held vertically. Access to the Perspex cell was by a coincident hole in the case of the absorptiometer, which could be covered by a sliding shutter when not occupied by a tube. Each culture vessel was marked so that it was placed in the Perspex cell in exactly the same position each time, with the aerating tube to the side and out of the way of the light path. The light path was cut down to a circular beam 6 mm. in diameter by means of a stop on the incident light side of the Perspex cell. This served to minimize any disturbing effect from the curved surface of the culture vessel and eliminate interference with the light beam by the aerating tube.

To correct for individual differences in the culture vessels the following procedure was adopted for measuring the optical density of cultures. The

Perspex cell containing water was used throughout as a fiduciary standard. The culture vessel containing the complete medium was placed in the absorptiometer and the small optical density (E_0') due to the tube and contents measured. The medium was inoculated with cells and the optical density again determined (E_0''), the difference between these two values giving the initial optical density of the culture (E_0). The value of E_0' for each tube was subtracted from all subsequent optical density readings of the culture to give the true value of E at time t.

Initial experiments largely confirmed the findings of earlier workers in that the optical density is approximately proportional to the number of cells per unit volume (cell concentration) up to an optical density of about 1·0. But, again as in the earlier work, it was found that there is no constant relationship from culture to culture between the optical density per unit thickness of culture and cell concentration. The relationship varies, amongst other things, with the physiological state of the cells and their content of chlorophyll. Preliminary experiments had demonstrated that a suspension of the organism shows no sharp absorption peaks in the visible when examined with a Unicam spectrophotometer. However, experience showed that the best results are obtained by using red filters (Chances OR 1). Investigations with various cell suspensions of equal cell concentration showed that there is a considerable variation in the ratio between the loss of light due to scattering and the loss of light due to absorption. By varying the optical system it was found possible to arrange that the optical density as measured approximated to that due to absorption alone. This was done by using a small stop on the incident light side of the Perspex cell and a large stop which exposed the whole of the active area of the photocell on the transmitted light side of the Perspex cell. The Perspex cell was fitted in the absorptiometer as close as possible to the photocell. These conditions ensure that nearly all the light which is scattered at an angle of less than 90° to the incident is received by the photocell. On the other hand, with the Perspex cell situated at some distance from the photocell and with a stop in the incident light the same size as that in the transmitted light path, all scattered light is lost and the optical density as measured includes the light lost by both absorption and scattering. The relationship between the optical density of a growing culture of *N. closterium* due to absorption and scattering, the optical density due to absorption alone, and the cell concentration is shown in Fig. 3.

It will be seen that a growth curve drawn from optical density measurements in the 'near' position approximates to that obtained by using cell concentrations. The rate of increase of this component of optical density is comparable to the rate of increase of cell concentration. This is not so with the curve drawn from the optical density measurements in the 'far' position. The relationship between the two optical density measurements and the cell concentration at various points during the growth is shown in Table I.

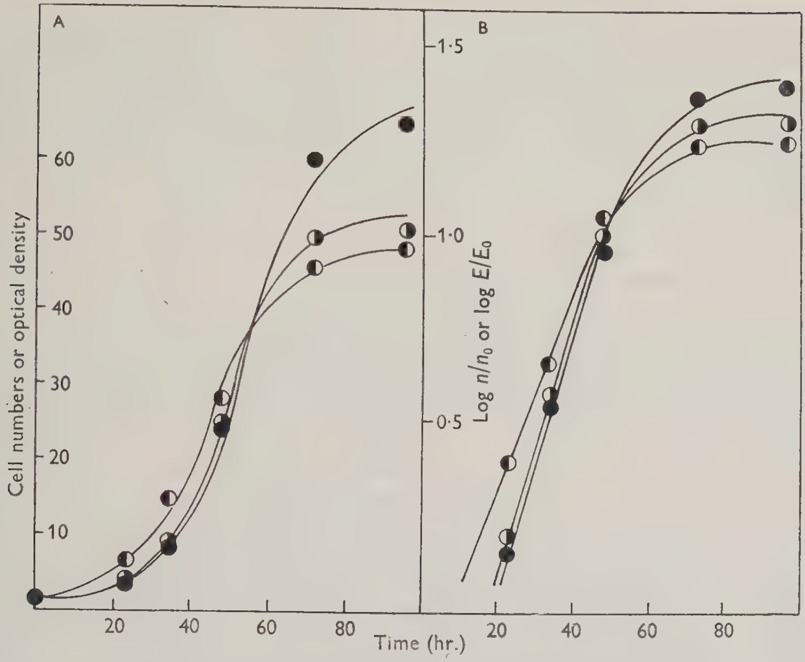


Fig. 3. The growth of a typical culture of *N. closterium* showing the growth curves obtained by using: (*a*) cell concentration, -●—●-; (*b*) optical density measurements in the 'near' position, -◑—◑-; (*c*) optical density measurements in the 'far' position, -◐—◐-. In A the ordinates represent arbitrary values of cell concentration and optical density calculated as follows: cell concentration, numbers per ml. × 10^{-5}; optical density in the 'near' position, $E \times 2{\cdot}5 \times 10^2$; optical density in the 'far' position, $E \times 3{\cdot}7 \times 10$. Optical densities measured using Chances OR1 filters. In B are given the corresponding logarithmic plots.

TABLE I. VARIATION IN THE LIGHT SCATTERED PER CELL AND THE LIGHT ABSORBED PER CELL THROUGHOUT THE GROWTH OF A CULTURE OF *NITZSCHIA CLOSTERIUM*

	Ratio O.D. per 10^6 cells per ml.					
Optical system	0 hr.	23 hr.	34 hr.	48 hr.	72 hr.	96 hr.
Optical density in 'near' position (approximates to light absorbed)	0·040	0·046	0·044	0·042	0·035	0·032
Optical density in 'far' position (approximates to light absorbed + light scattered)	0·268	0·490	0·420	0·310	0·202	0·199

The amount of light scattered per cell varies greatly throughout a growth cycle. It increases markedly during the commencement of exponential growth. Thereafter it declines steadily until the onset of the phase of decreasing growth rate when it exhibits a rapid decrease. The significance of measurements of O.D.$_{\text{(scattered light)}}$ must remain speculative. However, the variations in the amount of light scattered per cell suggests that this optical property depends largely upon cell density rather than cell concentration. Unfortunately no suitable techniques for the determination of dry weight are available due to the salinity of the culture media and the small amount of cellular material present. However, the amount of light scattered per cell does at least correlate with the volume of the protoplasmic content of the cells as observed under the microscope.

In contrast, the amount of light absorbed per cell is much more constant throughout a growth cycle. This is perhaps somewhat surprising since it might be expected that the amount of red light absorbed would be greatly affected by the chlorophyll content of the cells. It is known that the chlorophyll content of the cells decreases markedly during the phase of decreasing growth rate especially in nitrogen-limiting cultures (Harvey, 1953). Some variation in the light absorbed per cell does occur over this period, but the effect is not as great as might be expected. In addition, considerable increases in the chlorophyll content occur during the initial stationary phase, but these seem to have hardly any effect upon the amount of light absorbed per cell. Cells grown at differing lighting intensities have markedly different chlorophyll contents (Harvey, 1953) and optical comparison of such cell suspensions of equal cell concentration give different $\text{O.D.}_{(\text{absorbed light})}$ values. Nevertheless, it seems that when the cultures are grown in identical lighting conditions and, providing iron is in ample supply (Rodhe, 1948), the variation in the component $\text{O.D.}_{(\text{absorbed light})}$ per cell due to variations in the chlorophyll content are largely outweighed by other more constant factors. With the organism used, the overall size of the frustule seems to vary little throughout a growth cycle. Possibly it is this part of the cell which is the major factor controlling $\text{O.D.}_{(\text{absorbed light})}$.

To summarize, measurements of $\text{O.D.}_{(\text{absorbed light})}$ provide a method of comparing the cell concentrations of cultures of the organism used. Comparisons must be made between cultures in the same physiological states, grown in identical lighting conditions, and cultured with an adequate supply of iron. It is perhaps unwise to apply the method to any but bacteria-free cultures of the organism because of possible interference by bacterial growth. The cells of the culture must be evenly distributed and this necessitates continual agitation throughout growth, since microscopical examination shows that very considerable agitation is needed to break up all the cell clusters in a culture which has grown undisturbed for any time. The formation of precipitates in the culture medium must be avoided. This may be difficult since precipitation which is undetectable to the naked eye can occur in the course of the growth of a culture in the media used.

It must be emphasized that these considerations apply only to cultures of *N. closterium* and may not be applicable to algal cultures in general. It is known that the chlorophyll per cell decreases markedly during the phase of decreasing growth rate, especially in nitrogen-limiting cultures (Harvey, 1953). During this phase, and the subsequent exhaustion phase, the optical density increases more slowly than the cell concentration, and in consequence there is no strict proportionality between the optical density and the cell concentration. During these phases of growth variations of up to 23 % have been observed in the cell concentration corresponding to a given optical density.

Growth Experiments

The Exponential Growth Phase

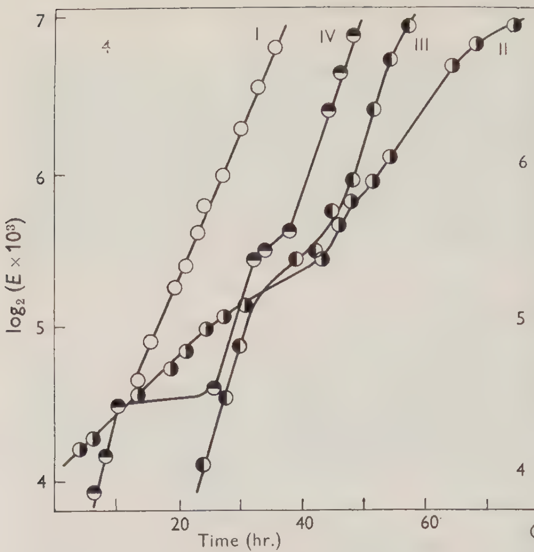
It has been found possible using the cultural conditions described to obtain regular exponential growth of *N. closterium*, and under these conditions the mean generation time has remained remarkably constant over many subcultures.

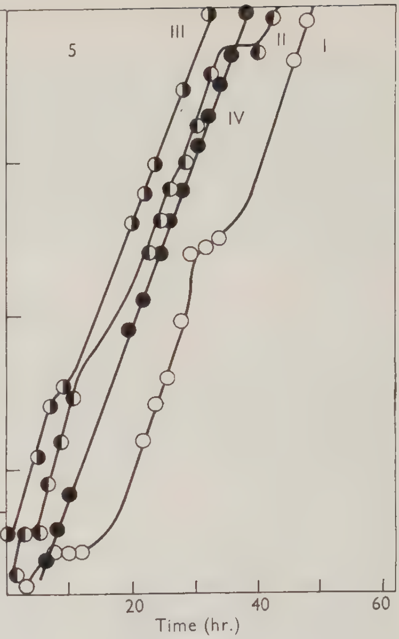
Effect of Changed Cultural Condition

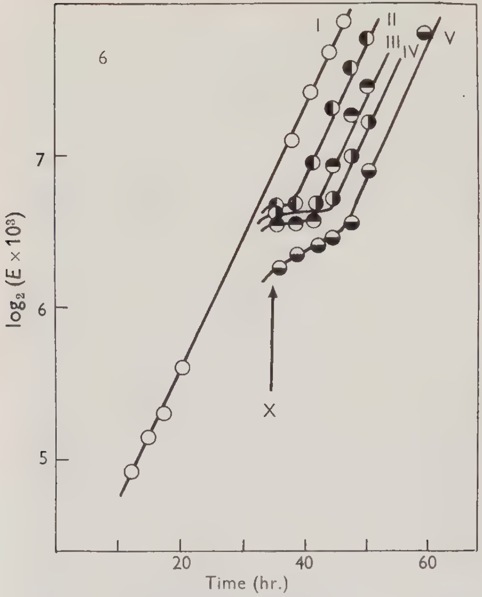
Before regular exponential growth can be obtained, the cells must be adapted to the physical and chemical conditions of the medium. Any change in these factors causes a period of irregular growth which may extend over many generations. Several factors, a change of which causes a period of irregular growth, have been investigated and will be discussed below. Fig. 4 shows some typical examples of irregular growth compared with the regular exponential growth obtained with cells adapted to the cultural conditions.

Curve I shows the typical regular exponential growth exhibited by cells adapted to the cultural conditions by previous growth in the normal basic medium enriched with additions of 0·6 mg. % nitrate-nitrogen and 0·6 mg. % phosphate-phosphorus per litre and grown with continuous illumination and aeration at 19° C. Curve II shows the nature of the growth obtained on subculture of these cells at 9° C. under otherwise identical conditions. Curve III is the growth obtained when cells adapted to the conditions of curve I are subcultured into medium containing 0·3 mg. % phosphate-phosphorus per litre, other conditions being unchanged. A similar irregular growth obtained on changing the cultural conditions is shown in curve IV. In this case cells grown on solid media were subcultured to the normal basic media under conditions identical to those under which the growth in curve I was obtained.

The irregular growth obtained on changing certain cultural conditions is generally characterized by periods during which no cell division occurs or of a much reduced division rate, alternating with periods of division at a greater rate than exhibited by adapted cells under the same conditions. That the phenomenon is essentially a lack of adaptation to changed cultural conditions is indicated by the observation that continued subculture in the new conditions finally leads to regular exponential growth being established.







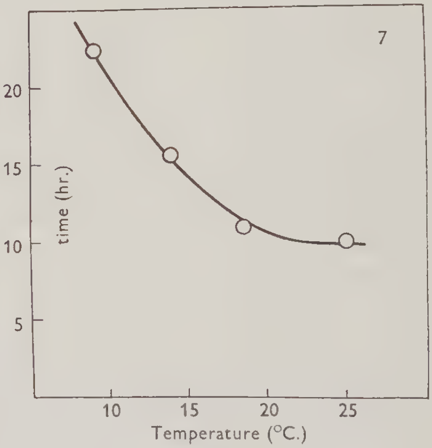


Fig. 4. Typical growth curves obtained with *N. closterium* by adapted (curve I) and by unadapted cells (curves II, III and IV). For details of culture conditions see text.

Fig. 5. The adaptation to changed cultural conditions. Parent culture grown on usual solid medium at room temperature in natural light from a north window and transferred to basic liquid medium with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration. Curves I–IV, second to fifth subcultures respectively.

Fig. 6. Replicate cultures in the basic medium with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus, grown to logarithmic phase at 19° C. with continuous illumination and aeration. After 34 hr. growth (*X*) cultures subjected to various periods of darkness and then re-illuminated.

Fig. 7. Variation of the mean generation time with temperature of cultures grown in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus with aeration and continuous illumination.

Fig. 5 shows the gradual adaptation to changed cultural conditions of cells which have been grown on solid medium in a north window in natural light at room temperature on subculture to basic liquid medium at 19° C. with constant illumination and aeration. The first subculture under the new conditions is not shown in the diagram. In this case five successive subcultures (together representing 20 to 25 divisions) had to be made before the cells became adapted to their new environment. This was one of the longest adaptation periods observed; sometimes as little as four divisions were sufficient to obtain regular exponential growth. In general the length of the adaptation period varies directly with the magnitude of the change in the cultural conditions. Once adaptation has been achieved the regularity of the exponential growth depends only upon the constancy of the prevailing cultural conditions.

When a culture of the organism is growing exponentially, the growth rate represents the resultant velocity of the whole series of reactions whereby cell substances are synthesized. The growth rate corresponds to the overall rate of a steady state system, the velocity constant of this system depending upon the resultant of the velocity constants of all the reactions involved. During such growth all the properties of the cell are constant, and any change in the cultural conditions may necessitate a change in the concentration of intermediates and enzymes. This re-organization may prevent regular exponential growth from being re-established immediately. It is possible that the irregular growths obtained are explicable in these terms.

The periods of very rapid growth alternating with periods of growth at a much reduced rate that are characteristic of unadapted cells suggest that the processes of cell division are in some way inhibited, whereas the synthesis of cell material may continue. In consequence, when cell division can occur it does so at a more rapid rate than normally by utilizing intermediates that have accumulated during the period of low division rate. The periods of rapid growth are soon succeeded by growth at the normal or at a suboptimal rate, presumably as the factors controlling cell division again come into play.

Effect of Dark Periods

The lighting conditions were such that the cells can be considered light-saturated with respect to growth. Under these conditions, periods of darkness cause an almost complete cessation of growth (Fig. 6). In the typical experiment shown re-illumination after up to 12-hr. periods of darkness allows an immediate resumption of regular exponential growth at the same rate as before. The experiment shown in Fig. 6 was performed with quintriplicate cultures all inoculated at the same time. The variations in cell concentrations after 36 hr. in cultures I, II, III and IV are in the same ratio as, and explicable by, variations in the size of the inocula. This is not so with curve V, the growth of which was apparently retarded by some unknown factor. It will be seen that during the period in the dark this culture exhibited a slow but marked growth until the cell concentration reached a value comparable with that of the other cultures before the illumination was stopped. It would seem that if growth is held back by inhibitory factors or deficiencies which primarily affect metabolic channels other than those involved in photosynthesis, some cell division can occur in subsequent dark periods. Presumably this occurs by utilization of 'excess' carbon skeletons formed during prior periods of photosynthesis.

It is interesting to compare these results with those obtained in similar experiments by Rodhe (1948). He observed a decrease in the optical density of cultures during dark periods and an initial 'growth' on re-illumination at a very rapid rate. This gradually dropped to a much slower rate. In the experiments reported in this paper no such effects during or after dark periods have been observed. There must have been respiratory losses during the dark periods, and the reason that these did not show up in optical-density measurements during the present studies can only mean that the optical density as measured is a function of cell concentration rather than cell density. On the other hand, it seems that Rodhe's optical-density measurements must have been a function of cell density. If this were so, the more rapid 'growth' he observed after dark periods could represent an initial rapid synthesis of cell material only, the division rate of the cells remaining unaffected.

Effect of Temperature

The exponential growth rate depends upon the temperature. The results of a preliminary investigation on the variation of growth rate with incubation temperature is shown in Fig. 7. The mean generation time falls rapidly with increasing temperature, reaching a minimum for these cultural conditions at about 20° C. Further increases in temperature have little effect on growth rate but no inhibitory effect is observed up to 25° C. All inocula for the experiments summarized in Fig. 7 were taken from a parent culture grown in media of identical composition at 19° C. A 10° C. change in temperature causes a period of irregular exponential growth which lasts for about four divisions. No adaptation period is required on subculture from 19 to 14° or 25° C. Between 9 and 20° C. the Arrhenius Law is approximately obeyed as might be expected. It is to be expected that further increase in temperature above 25° C. will lead to a rapid increase in the mean generation time followed by a complete cessation of growth resulting from denaturation of proteins and disorganization of the cell.

Effect of Carbon Dioxide Tension

For regular exponential growth to continue, chemical as well as physical conditions must be constant. Under normal cultural conditions the concentration of nutrients is not constant due to their continued utilization during growth, and division is stopped soon after the supply of one or more nutrients becomes exhausted. In the absence of aeration, the rate of diffusion of carbon dioxide into sea-water medium at a pH of about 8·6 is sufficiently slow to cause the supply of carbon dioxide for photosynthesis frequently to become limiting. This is clearly shown in Fig. 8 in which typical growths obtained with and without aeration are shown. It will be seen that, in the absence of aeration, the growth rate is soon affected and quickly drops almost to zero. In unaerated cultures the pH may rise to a value of 9·4 or higher before growth is stopped. The inhibition of growth in the unaerated culture is immediately reversed when aeration is recommenced.

It is apparent that aeration is necessary in cultures of the cell concentration used if the supply of available carbon for photosynthesis is not to drop below growth saturating levels. However, at least up to cell concentrations corresponding to a limiting nutrient concentration of 1·0 mg. % nitrate-nitrogen, it seems that bubbling with air will keep the concentration of available carbon sufficiently high to maintain regular exponential growth. A limiting nutrient concentration of 1·0 mg. % nitrate-nitrogen corresponds to a cell concentration of about 15×10^6 cells per ml. pH values in the region of 9·0 often occur in the later stages of regular exponential growth in aerated cultures. It is impossible to say from these results whether growth in non-aerated cultures is primarily inhibited by a shortage of available carbon or by the adverse effect of high pH. There is no evidence to suggest that at these growth rates and cell concentrations it is necessary to increase the partial pressure of carbon dioxide in the aerating gas to prevent this nutrient from becoming rate limiting. However, utilization of available carbon is sufficiently rapid to cause the pH to rise, and it may be advisable sometimes to use a higher partial pressure of carbon dioxide to obtain greater control of pH.

Effect of Other Nutrients

For maximum growth rate the concentration of the nutrients other than carbon dioxide must be such that all enzymic and other active centres are saturated with their respective substrates. No effect on the initial growth rate can be observed by reducing the nitrate-nitrogen concentrations or the phosphate-phosphorus concentrations down to 10 μg. %. Similarly, increases in the concentration of iron or manganese have no effect on the exponential growth rate. Consequently, it seems that the concentrations of these substances normally used are sufficient to saturate all active centres. It would be expected that, as the exponential growth rate becomes dependent upon a nutrient concentration at lower values, a hyperbolic relationship will exist between nutrient concentration and growth rate. However, special methods will be needed to determine the growth rate at these very low nutrient concentrations, since the total growth under these conditions is insufficient to permit an accurate determination of growth rate by the techniques used in the present studies.

THE TOTAL CROP

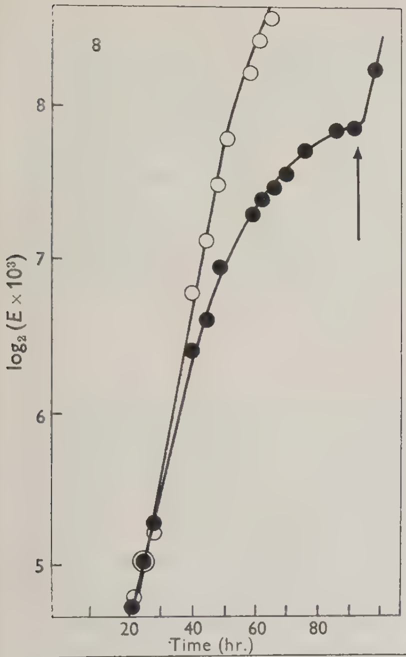
The Onset of the Phase of Decreasing Growth Rate

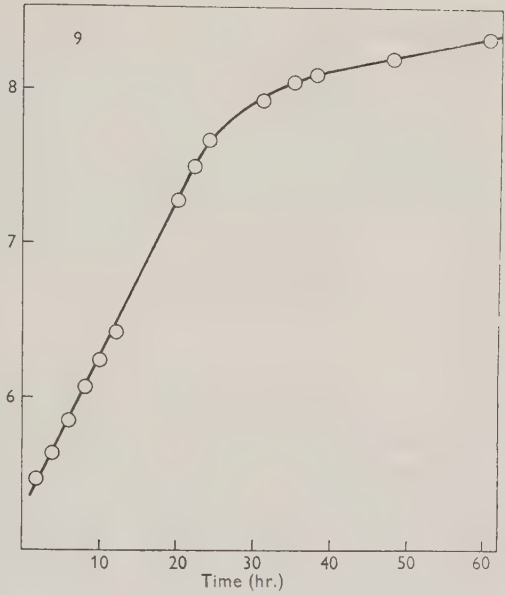
The total growth in any medium will be limited by the onset of one of several conditions. Amongst the factors which may cause cessation of exponential growth and the onset of the phase of decreasing growth rate, Monod (1949) lists the following: (i) the exhaustion of an essential nutrient, (ii) the accumulation of inhibitory metabolic products, and (iii) changes in the ionic equilibrium of the medium caused by the growth of the organism, for example pH. The culture conditions outlined above include an adequate supply of carbon in photosynthetically available form and thereby some degree of control of pH and there is no evidence that the supply of iron or manganese is limiting. The total growth should therefore be controlled only by the initial concentration of either nitrate or phosphate. Fig. 9 gives the latter part of a typical growth curve, showing the gradually decreasing growth rate typical of the exhaustion of a nutrient. The culture in this experiment was adapted to the prevailing conditions. It may be demonstrated that the decline of exponential growth is due to exhaustion of nitrate or phosphate since further additions of the limiting nutrient allow a resumption of growth. In this particular case the limiting nutrient was nitrate, but similar curves may be obtained in phosphate-limiting cultures. It will be seen that the growth rate gradually decreases but does not finally become zero. The subsequent slow regular increase in the optical density of such cultures continues for a considerable time and has been followed for as long as 72 hr. after the onset of this phase. Cell counts have been conducted on cultures exhibiting this phenomenon, and the slow increase in optical density indeed represents a continued slow division. The rate of cell division during this phase depends to some extent upon the cell concentration, there being a tendency for it to be greater with higher cell concentrations.

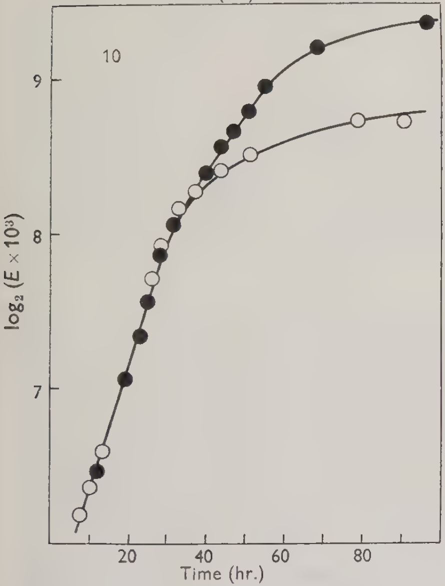
This slow regular increase of cell concentration after the phase of decreasing growth rate may be due to either the continual addition to the media of limiting nutrient via the aerating gas (e.g. ammonia), or to autolysis of senile cells regenerating limiting nutrient in a form available to the remaining viable cells. Because of this slow cell division it is considered undesirable to call this final phase the stationary phase. It will therefore be referred to as the exhaustion phase.

Adaptation to Increased Limiting Nutrient Concentration

In experiments in which the growth of the diatom has been followed in cultures containing an adequate and constant amount of phosphate and varying amounts of nitrate another form of adaptation to changed cultural







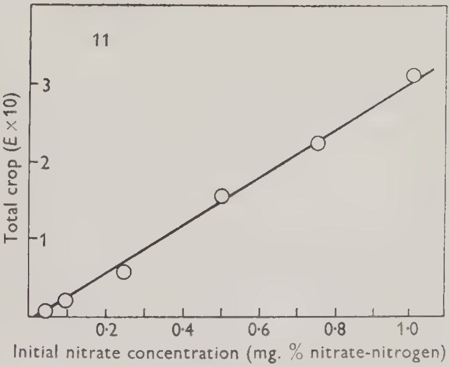


Fig. 8. Growth of *N. closterium* in the basic medium with additions of 1·2 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus with continuous illumination at 19° C. -○—○-, culture aerated; -●—●-, unaerated. Arrow indicates aeration restarted.

Fig. 9. Growth in basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus with continuous illumination and aeration at 19° C. showing the decrease in growth-rate caused by nitrate exhaustion.

Fig. 10. Growth in basic medium with additions of 0·3 mg. % phosphate-phosphorus with continuous illumination and aeration at 19° C., with varying amounts of nitrate as limiting nutrient. -○—○-, growth of parent culture with 0·6 mg. % nitrate-nitrogen as limiting nutrient. -●—●-, growth of subculture with 1·2 mg. % nitrate-nitrogen as limiting nutrient.

Fig. 11. The variation in the total crop when grown in the basic medium with additions of 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration and with varying initial amounts of nitrate as the limiting nutrient.

conditions has been observed. Fig. 10 shows a typical growth curve obtained when cells are grown in a nitrate-limiting medium to which they are adapted and the growth curve obtained when these cells are subcultured in the exponential phase to medium containing twice the amount of nitrate but otherwise of identical composition. The initial growth of the subculture is identical with that of the parent culture. However, when the cell concentration of the subculture approximates to a value equivalent to the limiting nutrient exhaustion in the parent culture, the growth rate of the subculture suddenly falls and thereafter continues at a steady but lower rate until exhaustion of nitrate leads to the onset of the phase of decreasing growth rate.

The growth rate during this second slower phase of exponential growth is increased by more efficient aeration and at lower cell concentrations the effect often does not occur at all. On the other hand, increasing or decreasing the carbonate alkalinity of the medium by additions of acid or alkali has no effect on the point of onset or the growth rate during this second exponential phase. Further subcultures in the higher nitrate concentration, however, finally allow exponential growth to continue at the initial rate until nitrate or carbon dioxide becomes limiting. Some evidence has been obtained that the addition of glucose at a concentration of 5·0 mg. % delays the onset of the point of inflexion on subculture of cells to a medium containing an increased limiting nitrate concentration.

The observation that adaptation must occur before uninterrupted exponential growth will extend to the expected cell concentration when the amount of a limiting nutrient is increased (Fig. 10) is unexpected. It seems most probable that this phenomenon represents an adaptation to enable cells to grow at an optimal exponential rate either at lower concentrations of photosynthetically available carbon or at a higher pH.

Relationship of Total Crop to Initial Concentration of Limiting Nutrient

With cells adapted to the limiting nitrate concentration, and with adequate amounts of phosphate and carbon dioxide available, the exponential phase continues to a cell concentration proportional to the initial nitrate concentration (Fig. 11). Due to the fact that the exponential phase is followed by a phase of gradually decreasing growth rate and since in the exhaustion phase the growth rate is not zero, it is impossible to determine accurately the cell concentration at which exponential growth ceases or the cell concentration that represents the crop due to a known addition of nitrate. The results shown in Fig. 11 are expressed in terms of an arbitrary value for the total crop. This has been defined as the cell concentration at the intercept when the exponential growth and the exhaustion phase are extrapolated to meet. Corrections must also be applied for the amount of available nitrogen added with the inoculum and the amount in the sea water used for making up the media. Providing this is done the crop is directly proportional to the initial concentration of the limiting nutrient. It is independent of the growth rate and of the inoculum size.

The results described above are for nitrate-limiting cultures. If the concentration of phosphate is made limiting analogous results are obtained with respect to the relationship between total crop and initial concentration of the limiting nutrient. In general, the total crop produced by a given limiting concentration of phosphate-phosphorus is equivalent to the total crop given by ten times this amount of nitrate-nitrogen.

The Initial Phases of Growth

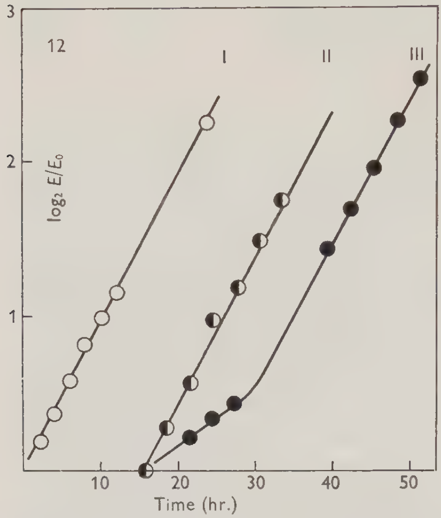
Effect of Limiting Nutrient Exhaustion

Providing a sufficiently large inoculum is used, cells subcultured from the exponential phase into fresh medium of identical composition immediately continue growth at the same rate. In contrast, cells which are subcultured from the exhaustion phase do not grow immediately but exhibit an initial stationary phase. Fig. 12 shows the growth of inocula subcultured (*a*) from the exponential phase, (*b*) from the early exhaustion phase, and (*c*) after storage in the exhaustion phase for 4 days. The growth curves show an increasing lag time (as measured by the intercept of exponential growth on the time axis) related to the state of exhaustion of the parent culture. In addition, cells which have been stored in the exhaustion phase for several days show an initial phase of slow growth between the initial stationary phase and the onset of growth at the optimum exponential rate. Consequently, if lag time for such cultures is measured as above it includes a period of 'apparent lag' during which some cell division is occurring.

The variation of lag time with the condition of the cells of the inoculum is shown in Fig. 14. The upper part of the figure shows the growth curve of the parent culture which was inoculated with nitrate-exhausted cells. The lower part of the figure shows the lag times observed in daughter cultures in medium of the same composition inoculated at intervals with samples from the parent culture. The cells in each sample were sedimented by centrifuging and resuspended in fresh medium to avoid carrying over any old culture medium with the inocula. The size of each inoculum was adjusted so that each daughter culture initially had approximately equal cell concentrations.

Cells subcultured during the initial stationary phase of the parent culture show a decreasing lag, but this does not become zero until the cells of the parent culture have been growing for some hours. Throughout the exponential phase of growth cells show no lag on subculture, but the lag time rapidly increases as the cells of the inoculum pass into the phase of decreasing growth rate. The lag continues to increase rapidly for the first part of the exhaustion phase. The rate of increase in lag time then declines, and after about 30 hr. in the exhaustion phase its rate of increase reaches a steady value.

The length of the lag period is clearly a function of the previous history of the cells and the long lags produced when the cells of the inoculum have been stored in the exhaustion phase for a long time must presumably be associated with a large number of reactions. Included in these must be the restoration of deficiencies caused by exhaustion of the limiting nutrient. There is considerable evidence to show that algal cells exhibit cell division after complete limiting nutrient utilization with the production of 'deficient' cells (Ketchum, 1939; Harvey, 1953). Comparison of measurements of growth by $\text{O.D.}_{\text{(absorbed light)}}$ and $\text{O.D.}_{\text{(absorbed light+scattered light)}}$ in the early phases of growth correlate well with the assumption that the initial stationary period consists of an increase in cell density, but not cell concentration.



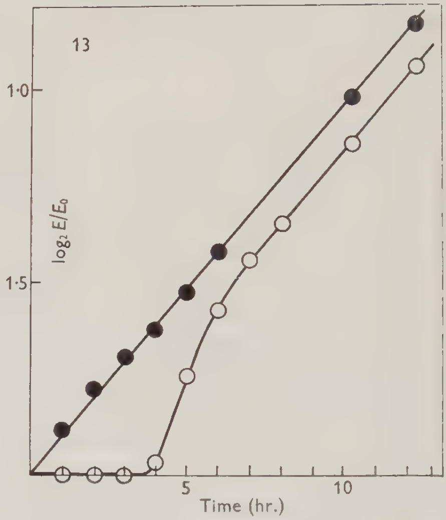


Fig. 12. Initial growth of *N. closterium* in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration. The cells of the inocula were adapted to these conditions by previous culture in the same media. The inoculum was of cells (I) from the exponential growth phase, (II) from the early exhaustion phase, (III) held with continuous illumination and aeration at 19° C. for 5 days in the exhaustion phase.

Fig. 13. Initial growth in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration on subculture to fresh medium. -●—●-, large inoculum. -○—○-, small inoculum. The cells of the inocula were adapted to the cultural conditions by previous subculture.

The initial stages of suboptimal growth obtained after long storage in the exhaustion phase (Fig. 12) might be caused by a proportion of the cells being capable of division at suboptimal rates. The magnitude of the effect tends to increase with increasing age of the inoculum as would be expected if this were so. The addition with the inoculum of a number of totally non-viable cells would not produce this type of growth.

Effect of Inoculum Size on Initial Growth

Cells subcultured during the exponential phase of growth show no lag if a sufficiently large inoculum is used. However, if smaller inocula are used a short lag is sometimes produced (Fig. 13). Close examination of the initial growth of subcultures using small inocula shows that the true division lag is greater than that obtained by extrapolating the normal exponential growth to $E/E_0 = 1$. This is due to an initial period of more rapid growth, as shown in Fig. 13.

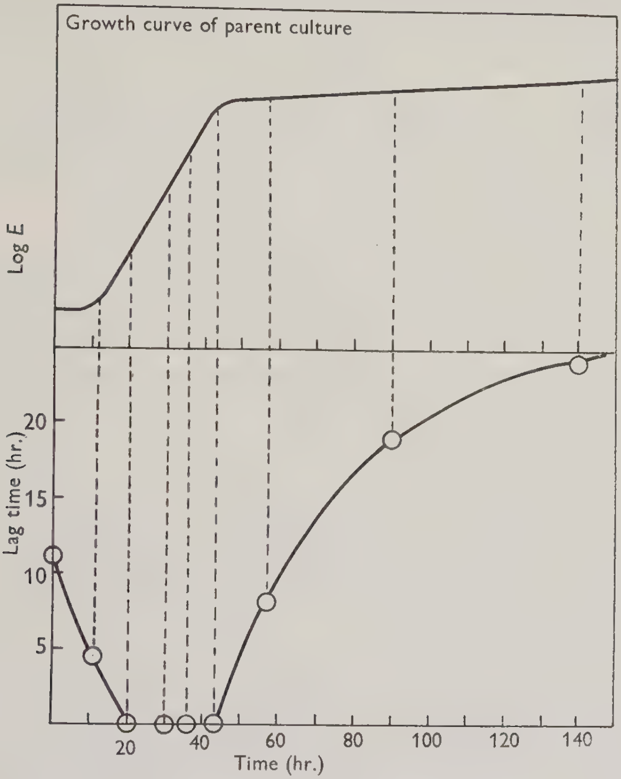


Fig. 14. Variation of the lag time (in the basic medium with additions of 0·6 mg. % nitrate-nitrogen and 0·3 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration) of cells of *N. closterium* with the age of the inoculum. The cells of all inocula were adapted to the cultural conditions by previous subculture.

All the results on the determination of lag reported above were obtained by using inocula of cells separated from the old culture medium by centrifuging. The more normal method of subculture is to transfer a few ml. of the parent culture to the new media and some old culture medium is therefore added with the cells of the inoculum. Comparison of the lag times observed when old culture medium was or was not transferred with the cells of the inoculum has shown no appreciable difference between the subsequent lags and growths produced when exhausted cells were used as an inoculum. If the subsequent lags and growths obtained using small inocula on subculturing cells from exponential growth to fresh medium and into re-enriched cell-free culture medium taken from an exponential culture are compared, somewhat irregular results are obtained. Nevertheless, these results do give a strong indication that under some conditions at least cells show less lag (of the type shown in Fig. 13) when subcultured into re-enriched old culture media than they exhibit on inoculation into new medium. It therefore seems possible that the lack of any observed effect on the addition of a small amount of old culture medium with the inoculum may be due to an insufficient amount being carried over. Cells which are growing exponentially, though at a reduced rate due to a higher limiting nutrient concentration than the one to which they are adapted (as shown in Fig. 10), often show a lag of 4–5 hr.

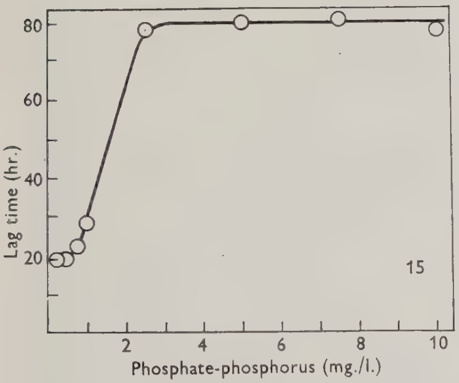
It is interesting that cells subcultured during the early part of exponential growth show a short lag. Taken in conjunction with the results obtained with different-sized inocula and with the effects on lag of old culture media, these observations seem to find explanation only in the assumption that some diffusable factors from the cells themselves are necessary for optimum exponential growth. The lags and subsequent more rapid initial growth rate observed when small inocula are used (Fig. 13) would make it appear that these factors are most directly involved in cell division than in the synthesis of cell material. Thus, with small inocula, the increase in cell density may continue normally, whereas cell division is inhibited for some time. When cell division can start, it is able to proceed more rapidly than normal for a short time until it is limited by the rate of increase of cell density, which presumably normally limits the rate of cell division.

Apart from these observations, which suggest that diffusable factors from the cells themselves may be involved in growth, no evidence has been found for the production by the cells of inhibitory substances which tend to limit growth. The production of such factors has been demonstrated with *Chlorella* by Pratt (1940) and his associates and for a species of *Nitzschia* by Denffer (1948). It remains possible that the 'adaptation' of *Nitzschia* cells to an increased concentration of a limiting nutrient may involve an adaptation to a higher concentration of a diffusable inhibitory factor, produced by the cells themselves.

Lags Induced by Varying the Initial Phosphate Concentration

All the lags considered up to now are exhibited by cells adapted to the initial nutrient concentration of the medium. Subculture to widely different nitrate-nitrogen concentrations of nitrate-exhausted cells has no effect on the lag times produced. However, if exhausted cells which have been grown in phosphate-limiting media are subcultured to new medium with an increased phosphate concentration longer lag times are produced (Fig. 15). It will be seen that the lag time increases with increasing phosphate concentration up to about a fourfold increase. Thereafter further increases in the initial phosphate concentration have little effect. Additions of phosphate in excess of 1·0 mg. % phosphate-phosphorus usually cause precipitation to occur. In some cases the initial growth in high phosphate concentrations is irregular.

A preliminary investigation of the phosphate exchange occurring during the long lag phase produced if phosphate deficient cells are subcultured into high phosphate concentrations shows that a complicated situation exists. The inorganic phosphate in the medium was estimated by Harvey's method (1948). The culture containing 0·062 mg. % phosphate-phosphorus took up practically the whole of this during the lag phase. A slight increase of the phosphate in the medium occurred after about 160 hr. when the culture was in the exhaustion phase. This is presumably due to autolysis. The cells subcultured in 0·24 mg. % phosphate-phosphorus took up 0·16 mg./100 ml. of culture in the first 30 hr. Growth did not start in this culture until after nearly 90 hr., during which time about 0·105 mg./100 ml. of the phosphorus originally taken up by the cells reappeared in the medium. When growth finally started in this culture



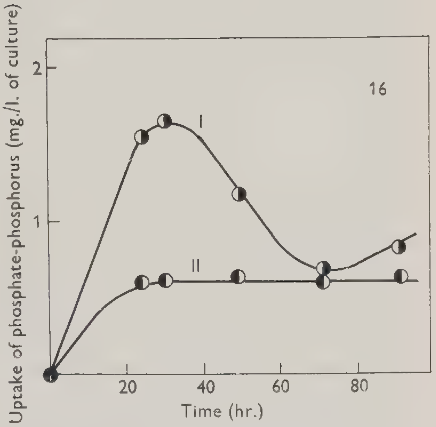


Fig. 15. Variation in the lag time of cells of *N. closterium* when grown in a medium containing 1·2 mg. % nitrate-nitrogen and 0·05 mg. % phosphate-phosphorus at 19° C. with continuous illumination and aeration and subcultured from the exhaustion phase under the same conditions of lighting, temperature and aeration into medium containing identical amounts of nitrate and varying amounts of phosphate.

Fig. 16. Uptake of phosphate by two cultures of equal cell concentration inoculated with cells from the exhaustion phase of a phosphorus-limiting culture grown in basic medium containing 1·20 mg. % nitrate-nitrogen and 0·06 mg. % phosphate-phosphorus. Curve I, basic medium with additions of 1·20 mg. % nitrate-nitrogen and 0·24 mg. % phosphate-phosphorus. Curve II, basic medium with the same addition of nitrate and 0·062 mg. % phosphate-phosphorus. Culture held at 19° C. with continuous illumination and aeration. Phosphorus estimations conducted on suitable samples after removal of the cells by centrifuging.

the phosphorus content of the cells was therefore approximately the same as at the end of the lag phase in the cells subcultured to a lower phosphate concentration. Phosphate uptake recommenced in the culture in high phosphate as soon as growth started and continued until at 160 hr. the whole of the phosphate had been taken up.

The prolonged lag produced on subculture to high phosphate concentrations is restricted to exhausted cells grown in a limiting amount of phosphate. If the exhausted cells have been grown in a limiting amount of nitrate and subcultured to high phosphate concentration, irregular initial growth of the type shown in Fig. 3 is usually obtained. In contrast, if cells growing exponentially in 0·05 mg. % phosphate-phosphorus are subcultured into an increased phosphate concentration of up to twenty times that of the parent culture there is no detectable effect on the lag time or subsequent exponential growth.

The very long lags produced when phosphate-deficient cells are subcultured to high phosphate concentrations (Fig. 15) must be due to other factors being superimposed on those discussed above. If sufficient phosphate is supplied it is taken up in quantities far in excess of the amount required to make good the phosphate deficiency of the cells and allow the resumption of cell division (Fig. 16). It seems that this excess phosphate completely disorganizes the cell and that cell division cannot start until this excess has been returned to the medium. It remains to be seen in what form the phosphate is returned to the medium; labile organic phosphate compounds would be estimated as inorganic phosphate by the methods used.

Discussion

The techniques developed during the present studies have made possible quantitative measurements of the growth of cultures of *Nitzschia closterium* forma *minutissima*. Regular exponential growth can be obtained which within certain limits is independent of the initial concentrations of the main nutrients and which varies with temperature according to the Arrhenius Law. The total crop produced is directly proportional to the initial concentration of a limiting nutrient and is independent of the growth rate. Control of experimental conditions has made possible a high degree of quantitative repeatability from culture to culture.

The fact that growth can be obtained which obeys a simple law suggests that under the defined conditions the basic medium used is suitable for quantitative growth studies. It is possible to predict accurately and control the rate of exponential growth under a given set of conditions and to control its extent by varying the initial concentration of a nutrient known to be limiting. The lag exhibited by an inoculum may be predicted from a knowledge of the previous history of the cells.

On the other hand, in sea-water medium, the limits within which such studies are possible are rather narrow. Due mainly to difficulties in the supply of photosynthetically available carbon, the culture used must not increase above a rather low cell concentration. Even so, in cultures aerated with air the pH rises rapidly during optimal exponential growth. This is not ideal and some way of controlling the pH would be advantageous. This is especially important since for many purposes it is desirable to work at higher cell concentrations. The use of a higher partial pressure of carbon dioxide in the aerating gas may in any case be necessary with higher cell concentrations, and an attempt should also be made to develop a medium, suitable for marine algae, that is buffered by a component not utilized during growth. This will necessitate changes in the major ionic constituents since the high concentration of calcium makes sea water a very inflexible basis for media.

Similar factors may produce troublesome precipitation during the growth of cultures. This precipitation is not obvious to the naked eye, but shows itself by anomalous optical-density readings and often in sudden fluctuations in the pH of the culture. No way has been found of controlling this type of precipitation, and its occurrence has led to many cultures having to be rejected. Similarly, sea water is often difficult to sterilize by autoclaving due to precipitation occurring. Filtration techniques for sterilization have not been widely investigated, but it seems probable that, owing to the very low concentration of many ions in sea water, such techniques may lead to difficulties resulting from exchange phenomena and adsorption. The procedure used in the present studies, however, of diluting the sea water before autoclaving, mitigates the precipitation. A preliminary survey (Miss D. Ballantine, private communication) shows that many marine unicellular algae will tolerate this decreased salinity and the technique may be generally applicable.

It is difficult to obtain uniformity from one batch of sea water to the next. The 'ageing' process assists qualitative uniformity but it may produce widely different quantitative compositions, due, for example, to the release of available nitrogen by the bacterial decomposition of organic matter. When working at low cell concentrations these increases are large compared with the small amounts of nutrient added.

This work was performed at the Plymouth Laboratory of the Marine Biological Association. I should like to express my thanks to the Director, Mr F. S. Russell, F.R.S., for making freely available all the facilities of the laboratory; to all the staff for their helpfulness, and especially to Dr Mary Parke and Mr F. A. J. Armstrong, and to the latter for carrying out the phosphate estimations. Finally, it is a great pleasure to thank Dr H. W. Harvey, F.R.S., for introducing me to the field, for advice in the construction of the absorptiometer, and for his continued interest and many stimulating discussions.

SUMMARY

Techniques are described for the culture of a bacteria-free strain of *Nitzschia closterium* forma *minutissima* under carefully controlled conditions. An investigation has been made of the absorption and scattering of light by cultures of the organism and a technique developed which enables an absorptiometric method to be used for measuring the growth of cultures.

Using the techniques described, the nature of the growth of the diatom has been studied in media made up by the addition to sea water of nutrient salts and trace elements. These studies have defined the limits within which such media may be used for quantitative growth studies and have indicated the limitations imposed on growth studies by media of this type.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ÜBER DIE SEEWASSERRESISTENZ VON MEERSTRANDPFLANZEN
[THE RESISTANCE OF SEA-SHORE PLANTS TO SEA WATER]

By **Richard Biebl**

Photogr. u. Forsch., Bd. 5, 1953, pp. 174–80

Some observations are described on the cell physiology of land halophytes from the rocky shore of Wembury, South Devon. Photographs of some of these plants are given. The observations on the osmotic value, permeability, and resistance to salt were carried out in different concentrations of sea water. Sea water is the best naturally balanced salt solution. Shore plants have a higher osmotic value and a greater resistance to sea water than glycophytes like plants of meadows and gardens. Also the permeability to sea water is different. R.B.

FURTHER WORK ON PAGURIDS AND THEIR COMMENSALS

By **L. R. Brightwell**

Proc. zool. Soc. Lond., Vol. 123, 1953, pp. 61–4

In September 1951 efforts were made at the laboratory to investigate reports from London Zoo Aquarium that *Nereis fucata* would eject the Vestlet Anemone from its tube, and even *Eupagurus bernhardus* from its borrowed shell. Anemones were not available, but by means of glass Buccinum shells it was established that two worms may force entry to the same shell, rendering it untenable for the crustacean. It was further noted that the Lesser Spotted Dog-fish and *Nereis fucata* between them may do much to provide homes for the common hermit. The fish bites off the whelk's head and foot, whilst the worm disposes of the visceral mass. The worm will also eat the abdominal portion of a dead hermit after the cephalothorax and legs have been eaten by almost any bottom-feeding fish.

Observations were made on the largely planktonic diet of *Eupagurus bernhardus*. When a *Calliactis* emitted a stream of ova, for over 2 hr. without pause, a hermit sat beside the anemone, and by its inhalatory respiratory current, very deliberately diverted large numbers of ova to its own interior.

It was found that quite frequently *Eupagurus bernhardus* will accept almost any refuge, even a straight glass tube, rather than leave its abdomen open to attack. This lends colour to Sinel's suggestion that the pagurids may have evolved from one of the so-called burrowing prawns, such as *Callianasa*.

L.R.B.

The Larva and Adult of *Polycitor crystallinus* Renier (Ascidiacea, Polycitoridae)

By **D. B. Carlisle**

Proc. zool. Soc. Lond., Vol. 123, 1953, pp. 259–65.

The anatomy of the adult and larva of this species are described and the differences from *Polycitor vitreus* (Sars) discussed in detail. A synonymy of the species is given, together with a summary of its known distribution, which is confined to the Mediterranean area. Its relationships with the Polyclinidae are discussed, and the suggestion is made that the Polycitorinae should be raised to full familial rank and separated from the Clavelinidae. D.B.C.

The X-organ of Crustacea

By **D. B. Carlisle and L. M. Passano**

Nature, Lond., Vol. 171, 1953, pp. 1070–1.

The X-organ consists of three types of cells at least. In Brachyura, where the sensory pore is poorly developed or wanting these three different types are commonly found together, usually at the proximal ventral corner of the medulla terminalis, but in Stomatopoda, Anomura, Natantia and *Dromia* the X-organ may be separated into two parts connected by a nerve. The three-cell types are large neurosecretory cells, smaller isodiametric cells, often containing each a single large inclusion, and oval or irregular 'concretions of concentric structure' which show no nucleus and may be products of secretion rather than cells. In most Brachyura all three types are associated into one localized organ, while in the other groups examined the first cell type forms a single group in the medulla terminalis, whereas the other two types of cells are located in a separate organ near the sensory pore. This organ is named the *pars distalis X-organi*, and that in the medulla terminalis is named the *pars ganglionaris X-organi*; the nerve connecting the two parts is the X-organ connective. These findings may serve to explain why X-organ extirpation has different results in different groups of Crustacea. D.B.C.

ON A SPOROZOON IN THE COELOMIC CORPUSCLES OF *PHASCOLOSOMA MINUTUM* KEFERSTEIN (SIPUNCULOIDEA)

By **D. Etherington**

Parasitology, Vol. 43, 1953, pp. 160–9.

A description is given of a sporozoon infecting the coelomic corpuscles of *Phascolosoma minutum.* The parasite undergoes schizogony in the haematids of the coelomic fluid; the schizonts are enclosed in tough envelopes. Two types of merozoites are produced; micromerozoites repeat the schizogonic cycle, while macromerozoites invade haematids, but develop into encysted bodies when the haematids are ingested by amoebocytes. In the discussion of the affinities of the parasite, it is suggested that the encysted bodies are gamonts, and that the parasite is related to the Haemogregarinidea. D.E.

NEW FORMS OF VISUAL PURPLE FROM THE RETINAS OF CERTAIN MARINE FISHES: A RE-EXAMINATION

By **E. M. Kampa**

J. Physiol., Vol. 119, 1953, pp. 400–9.

Dark adapted retinas of three marine fishes (plaice, *Pleuronectes platessa*; pollack, *Gadus pollachius*; and gurnard, *Trigla hirundo*), previously reported to possess unique photopigments (Bayliss, Lythgoe & Tansley, *Proc. Roy. Soc.* B, Vol. 120, 1936, pp. 95–113), have been shown to contain pigments with absorption spectra indistinguishable from that of frog rhodopsin.

The dark-adapted retina of the freshwater tench, *Tinca vulgaris*, has been shown to contain a pigment with an absorption spectrum characteristic of porphyropsin. The absorption spectrum of an extract of dark-adapted trout (*Salmo trutta*) retinas indicates that this anadromous teleost may utilize a mixture of rhodopsin and porphyropsin.

Differences between the methods used in the present work and those employed by Bayliss *et al.* are discussed. None of these seems sufficient to explain the differences between the two sets of results. E.M.K.

THE EFFECT OF ENUCLEATION ON THE DEVELOPMENT OF SEA-URCHIN EGGS. I. ENUCLEATION OF ONE CELL AT THE 2-, 4- OR 8-CELL STAGE

By **I. J. Lorch, J. F. Danielli and S. Hörstadius**

Exp. Cell Res., Vol. 4, 1953, pp. 253–62.

Enucleated blastomeres left in contact with one, three or seven normal blastomeres did not play any part in the development of the larva, except in so far as purely mechanical distortion was produced.

After an initial delay, during which asters and incomplete division furrows appeared and disappeared again, the enucleated cells divided rapidly into a number of spheroidal cells of various sizes. The final diameter of these cells ranged from 3 to 50 μ. The cells remained optically similar to nucleated cells of the same size and retained their semi-permeability for 3 days or more.

Enucleated cells were never seen to differentiate or participate in the formation of tissues or organs. The presence or absence of asters in the early stages made no difference to the final fate of the enucleated cells.

Thus it seems that the nucleus is essential for differentiation, though not for cell-division. The proximity of normally differentiating cells does not induce similar changes in the enucleated cells. I.J.L.

FURTHER STUDIES ON IONIC REGULATION IN MARINE INVERTEBRATES

By **James D. Robertson**

J. exp. Biol., Vol. 30, 1953, pp. 277–96.

In a study of ionic regulation in the blood or coelomic fluid of sixteen marine invertebrates, differences from a passive equilibrium with the external medium were found in all. This active regulation is most pronounced in *Sepia* and members of the decapod Crustacea, all of which have excretory organs capable of selective ionic excretion. Little regulation is shown by holothuroids or lamellibranchs. In a group of sixteen crustaceans, it was found that the magnesium of the plasma varied between 14 and 101 % of the value in the surrounding sea water. Those with high levels (e.g. *Maia*, *Dromia*, *Lithodes*) are slow-moving and inactive, those with low levels (e.g. *Squilla*, *Homarus*, *Pachygrapsus*) are capable of swift movement and are generally more active. J.D.R.

NEW TURBELLARIA PARASITES IN ECHINODERMS

By **Einar Westblad**

Ark. Zool., Ser. 2, Bd. 5, 1953, pp. 269–88.

Three turbellarian parasites are described, found in intestines of echinoderms at Plymouth. In all six turbellarian parasites were found in echinoderms:

ACOELA:

Gen. *Avagina* Leiper (1902, 1904).
    A. incola Leiper: in *Spatangus purpureus* (common).
    A. glandulifera Westblad, 1953: in *Spatangus purpureus*.

RHABDOCOELA:

Gen. *Umagilla* Wahl, 1910.
    U. forskalensis Wahl: in *Holothuria forskali* (common)
Gen. *Anoplodium* Ant. Schneider, 1858.
    A. tubiferum Westblad, 1953; in *Holothuria forskali.*
Gen. *Marcusella* Westblad, 1953.
    M. atriovillosa Westblad, 1953: in *Spatangus purpureus.*
Gen. *Syndesmis* W. Silliman, 1881.
    S. echinorum François: in *Echinus esculentus* (common).

In addition, *Fecampia erythrocephala* Giard 1886 was found in small specimens of *Carcinus maenes* from Rum Bay.

BOREOHYDRA SIMPLEX WESTBLAD, A 'BIPOLAR' HYDROID

By Einar Westblad

Ark. Zool., Ser. 2, Bd. 4, 1953, pp. 351–4.

MARINE MACROSTOMIDA (TURBELLARIA) FROM SCANDINAVIA AND ENGLAND

By Einar Westblad

Ark. Zool., Ser. 2, Bd. 4, 1953, pp. 391–401.

In these papers the following species have been recorded in the Plymouth area:

Boreohydra: at Plymouth (Hamoaze at a depth of *c.* 16 m., 26 July 1949).

Gen. *Macrostomum* O. Schmidt, 1848.
    M. appendiculatum (O. Fabr, 1826) Graff, 1905: Salcombe estuary, in July 1949.
    M. pusillum Ax, 1951: Yealm estuary, common in mud, in July 1949.
Gen. *Microstomum* O. Schmidt, 1848.
    M. hamatum Westblad, 1953: Plymouth, the harbour in black mud at *c.* 40 m. depth, in July 1949
    M. rubromaculatum Graff, 1882: Wembury, in tide pools, in July 1949

BOOK REVIEW

The Genus *Euglena*

By **Mary Gojdics**

Madison, University of Wisconsin Press, pp. viii + 268, 1953.

This is an admirable work, and although it is primarily taxonomic the first forty pages or so are devoted to a clear description of the organism's general biology and physiology. This part of the work is well done, and it is clear that the numerous papers connected with it have been carefully studied by the author.

The bibliography comprises some 300 references.

There are numerous illustrations including thirty-nine plates with very clear line figures.

In addition to the 'keys' the environment of each species is described. The importance of studying cultures grown from a single individual, as advocated by S. P. Chu, is advocated but not described.

Since *Euglena* is the standard type of an autotrophic flagellate it should loom largely in the teaching of general biology; and for teaching purposes as well as for those engaged in physiology alone a short description of actual culture technique would add further value to the work.

A. G. LOWNDES

J. Mar. biol. Ass. U.K. (1954) 33, 297–312
Printed in Great Britain

THE BIOLOGY OF *LIMACINA RETROVERSA*

By J. E. Morton
Department of Zoology, Queen Mary College, University of London

(Text-figs. 1–5)

The Plymouth Fauna List contains records of two genera of pteropods, *Limacina* and *Clione*. Of the first, *Limacina retroversa* (Flem.) is by far the better-known species, apparently occurring regularly at Plymouth in large numbers in townettings from outside, and sometimes inside, the Sound. It breeds at Plymouth from June to August, and Lebour (1932) has given a detailed account of its breeding and larval stages and has discussed its role in the plankton. The second species of *Limacina* at Plymouth is *lesueuri* (d'Orbigny), which has been observed from time to time since 1906, when it was very common. The last record in the Fauna is off the Breakwater in 1920. Of the gymnosomatous pteropods, *Clione limacina* Phipps is the only species regularly occurring. Lebour (1931) has described the life history of this form, and mentions February to August as its months of greatest abundance. Its breeding season is June to August. Another gymnosome, tentatively referred to as *Clionina longicauda*, is reported by Russell (1936), and from the specimen department at Plymouth Laboratory the writer obtained some preserved specimens, not easily identifiable, of a *Pneumodermopsis* taken locally; its species is perhaps *ciliata*, recorded by Massy (1917) from the Irish Coast.

In spite of its abundance and wide distribution, *Limacina* has not been carefully studied in the living condition. In Meisenheimer's long account (1905) of pteropod morphology, the functional aspect is rather understressed. Nor did Yonge (1926), in his account of feeding mechanisms in thecosomatous pteropods, deal with *Limacina*. A knowledge of this genus would, however, undoubtedly shed much light on the study of the larger and more specialized thecasomes. Hsiao (1939) has already worked on the sexual succession and life history of populations of *L. retroversa*; the present writer has completed an account of the genital system and reproduction of *L. bulimoides* from the South Atlantic. During the summer of 1952, when abundant and active material of *L. retroversa* was available at Plymouth, an opportunity was taken to examine its movements, mode of feeding, and the structure and action of the digestive system. From similar living material of *Clione limacina*, it is later intended to give an account of the structure and reproduction of this pteropod.

I am indebted to the Director and Staff of the Plymouth Laboratory of the Marine Biological Association for their kindness during my tenure of the

University of London table; and I have especially to thank Dr D. Atkins for first obtaining samples of living material for me, and for drawing my attention to its times of abundance at International Hydrographic Station E I.

Movements

Limacina swims with the aperture of the shell upwards and the surface of the foot widely exposed above. Its movements in a laboratory vessel consist of repeated upward migrations to the surface, describing a broadly spiral course, followed by a more rapid and direct descent during which the wings are out of action. In upward swimming the rhythmically flapping wings constitute a tractellum producing an eddy behind which the animal moves. The effective stroke appears to come about by the contraction of the sheet of muscle fibres running through the base of the ventral side of each wing, underlying the ciliated field, to be referred to below. At the resting stage, the dorsal muscle sheet of the wings would appear to contract and the thicker bases are reflected backwards against the shell to show their under-surfaces which are also ciliated. The expanded tips of the wings are transparent and non-muscular. They are held freely relaxed until a contraction of the dorsal muscle flicks the bases forward again. During descent the wings offer no resistance to the water and are held erect and close together above the animal, forming—when viewed from above—two planes at an oblique angle. The weight of the compact shell and body quickly carried the animal downwards. Fig. 1 illustrates the successive positions of the wings during swimming. From references in the literature to other Thecosomata (see, for example, Stubbings, 1937), it seems likely that the principal movements of these pteropods are in a vertical direction, with the surface of the foot widely exposed for feeding. In *L. bulimoides* there is evidence that this species performs regular diurnal depth migrations (Morton, 1954).

Food Collecting

Limacina is entirely a ciliary feeder. It apparently lives chiefly upon dinoflagellates and suitably shaped diatoms. When the Thecosomata became pelagic it is probable they had already lost the ctenidium which was used to produce food-collecting currents in ciliary-feeding prosobranchs. They rely to a much greater extent on the ciliated surfaces of the foot. Yonge (1926), in his account of feeding in the Cavoliniidae and Cymbuliidae, maintains that feeding is carried out entirely by the lobes of the foot, and the cilia at the bases of the wings. As we shall later discuss, it is clear that these families are a good deal more advanced in structure than the spiral-shelled Limacinidae, where it would appear that, in spite of the loss of the ctenidium, the mantle cavity is still the most important feature of the feeding apparatus.

We may first look at some external structures of *L. retroversa* (Fig. 2) of which the most prominent are the foot and the wings. The foot consists of

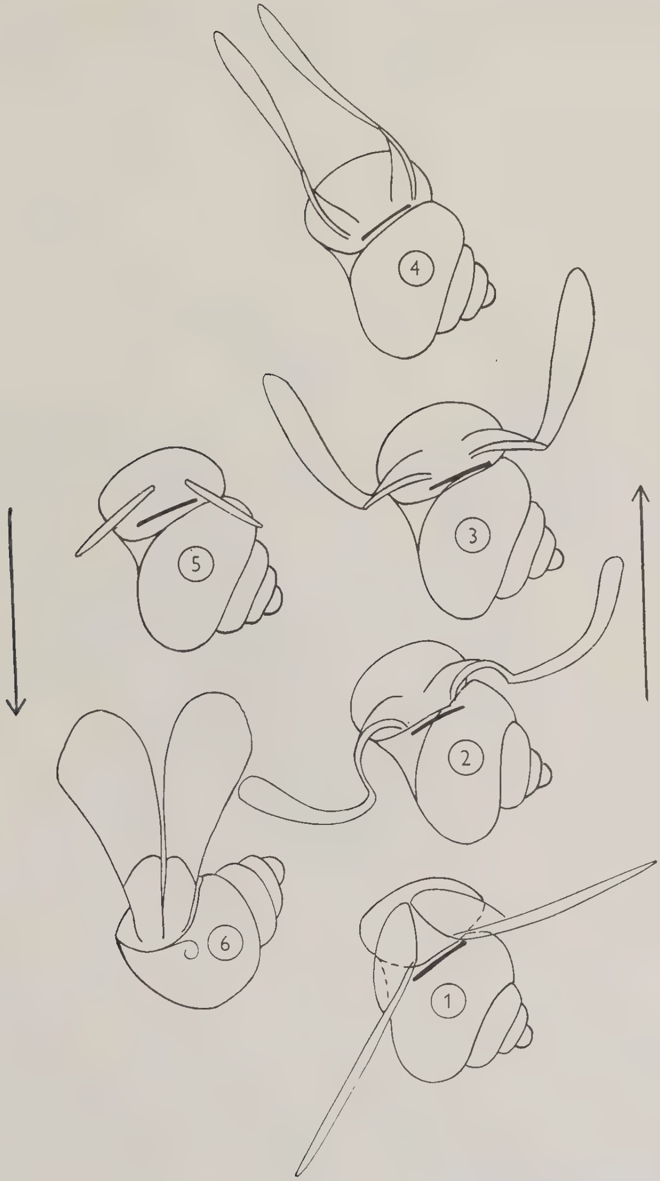

Fig. 1. *L. retroversa.* Outline drawings showing successive positions of the wings in swimming upwards (1–4), and in descending (5). 6 shows the wings held motionless and vertical, in a different view from 5.

a flat median lobe (M L) flanked by two narrower lateral lobes (LAT L). The median lobe is broadly triangular or heart-shaped, widest behind and carrying upon its back surface the transparent, chitinous operculum, golden yellow in colour. This is almost always retained in limacinids though, as Lebour (1932) points out, it may often drop off in older specimens of *L. retroversa*. The flat

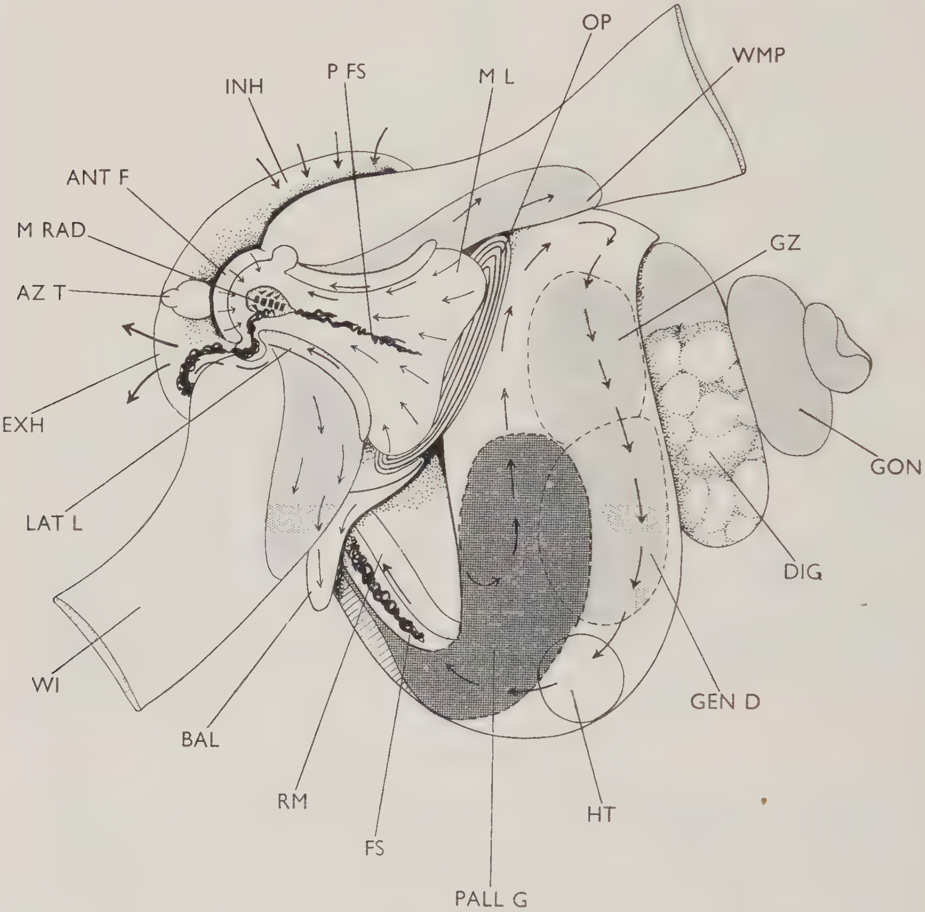

Fig. 2. *L. retroversa*, ventral view. Diagram showing the ciliary currents employed in food collecting. The surface of the foot and the ciliated portions of the wings are fully expanded, and the distal parts of the wings are omitted. The contents of the pallial cavity are shown as if transparent, the pallial mucus gland, the genital ducts and the stomach being represented by stippled areas. ANT F, anterior horseshoe-shaped fold; AZ T, azygous tentacle; BAL, balancer; DIG, digestive gland; EXH, exhalant side of pallial cavity; FS, food string within the pallial cavity; GON, gonad; GEN D, genital duct; GZ, gizzard; HT, position of the heart; INH, inhalant side of the pallial cavity; LAT L, lateral lobe of the foot; M L, median lobe of the foot; M RAD, mouth, with the radula; OP, operculum; P FS, food strings formed on the median lobe of the foot; PALL G, pallial mucous gland; RM, rectum; WI, wing; WMP, ciliated 'Wimperfeld' at base of wing.

surface of the median lobe represents the sole and is finely and uniformly ciliated, and covered with unicellular mucous glands. In front it narrows rapidly, and channels forward towards the mouth between the two lateral lobes which form straight or crescentic folds, bounding the sole on either side from the bases of the wings. These folds are dark brown or blackish in colour, and beneath them and along their edges are tracts of dense forward-beating cilia. These lead into a shallow funnel-shaped depression, enclosing the mouth, and bounded in front by a small, horseshoe-shaped fold (ANT F), lighter brown in colour. Outside the lateral lobes of the foot spring the paired swimming wings, diverging at right angles, one at either side. They expand slightly towards the free tips, which are thin and membranous and somewhat squarish. Each wing has two areas, a rather smaller triangular area at the base, widest posteriorly, and bearing cilia (referred to as the 'Wimperfeld' by Meisenheimer), and the longer free flaps which are colourless and translucent. The ciliated areas are thicker and firmer, and the contraction of their dorsal and ventral muscle sheets results in the movements causing locomotion. In front of the horseshoe-shaped fold bounding the mouth, the head bears a single azygous tentacle (AZ T) with a short terminal papilla inserted in a cylindrical base. Its unpaired condition is a peculiar and unexplained feature, which seems to be common to limacinids in general (Pelseneer, 1888).

The coiling of the visceral mass is sinistral so that the inhalant and exhalant openings of the mantle cavity are reversed from the position usual in gastropods. The pallial aperture is wide and unconstricted, but the chief point of entry of the water current is on the left side near to the posterior corner of the pallial opening. Here the rim of the mantle is encircled with dense cilia beating inwards; their action is so strong that the particles from the surrounding water from a distance of 5–10 mm. are rapidly cleared and drawn into the pallial cavity. Along the whole of the inhalant side, the inner surface of the mantle is clad with cilia. A short way behind the aperture it becomes glandular, with the appearance of a thick, broad shield of mucus-producing epithelium, which extends around the longer or concave side of the pallial cavity and reaches back to the pericardium on the right side. This is the 'shield' or pallial gland, mentioned by Pelseneer (1888) and the 'manteldruse' of Meisenheimer, and in its structure and histology it forms a very constant feature of all known Thecosomata. In position it has the same relations as the hypobranchial gland of prosobranchs, though in histology there seems to be no real similarity. Meisenheimer (1905) provides a simple figure of the 'manteldruse', and we may illustrate it again here for *Limacina* (Fig. 3, right side). The glandular cells are of uniform height (45 μ) and width (5 μ), and the superficial layer of the epithelium, to about one-fourth its depth, contains a row of ovoid mucous droplets, ready for secretion. The remaining cytoplasm after Bouin's fixation tends to break up into a number of vertical strands of darker staining secretion (brown in azan) while the large, binucleolate nuclei lie in a single row at the base of the epithelium. Ciliated cells are smaller and less frequent, about one to each pair of glandular cells. They appear as tiny wedges with dark nuclei, inserted between the tips of the glandular cells, but they have evidently a narrow drawn-out connexion with the basement membrane and seem to remain intact during secretion.

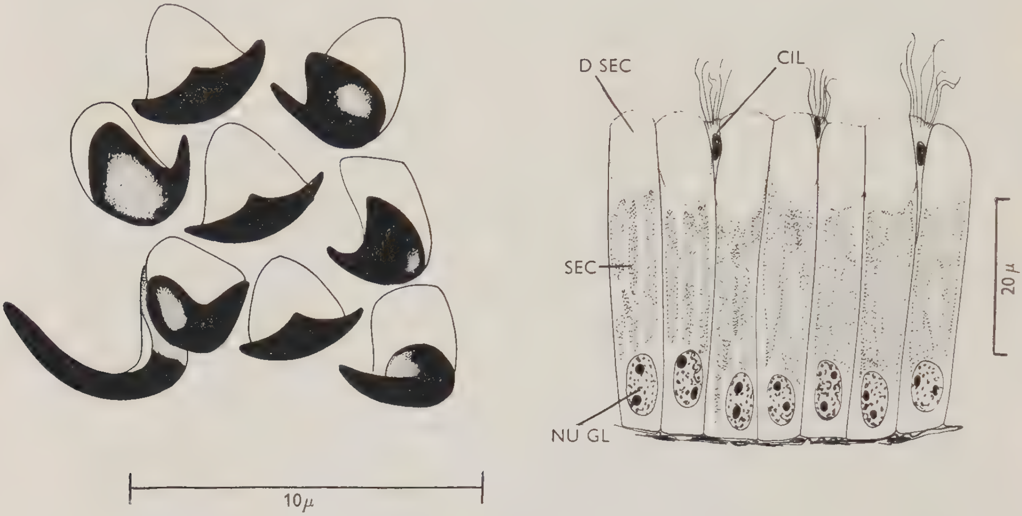

Fig. 3. *L. retroversa.* Left side. Three rows of radular teeth. A lateral mounted flat is shown in the lower row, left side. Right side. Epithelial cells of the pallial mucous gland. (Bouin's fluid, Heidenhain's azan). CIL, ciliated cell; D SEC, distal droplet of secretory material; NU GL, nucleus of gland cell; SEC, darker staining secretory material in the middle part of the cell.

The function of the pallial gland in *Limacina* is to provide mucus for compaction of the particles entering the inhalant side of the pallial cavity, and to carry forward a coherent string of collected particles along the right side of the cavity. Fig. 2 shows the course of the ciliary currents carrying particles into the pallial cavity. There is a strong inward stream on the left side to the tapered upper end of the cavity, with a sharp reversal at the top, and the return of collected material along the pallial glandular strip at the right side. This part of the pallial cavity in *Limacina*, extending far back alongside the stomach and anterior part of the digestive gland, calls to mind in a more elementary form the more elaborate pallial caecum of *Actaeon* (see Fretter, in press) and *Scaphander*, with inward and outward ciliary currents. *Limacina* itself would appear to require little help from ciliary currents in this region: the whole circulation is probably brought about chiefly by the action of the inhalant cilia lying farther forward. Only the lightest of particles seem to make the complete circuit of the pallial cavity. Coarser material, of the size of diatom frustules, constantly appears to drop out of the stream on to the pallial mucous gland, and to become bound up into the string that constantly moves forward on the right side. This string is carried by cilia along the surface of the glandular sheet (FS) and issues from the mantle cavity on the right side at the anteriormost part of the aperture. Its contents are regularly ingested. They make their way to the funnel containing the mouth, across a tiny, saddle-shaped depression, between the right lateral lobe of the foot and the horseshoe lobe. There is a similar depression on the left side, which was however never seen to be crossed by a food string. At the bottom of the funnel the narrow radular ribbon is thrust from the mouth, and the tiny chitinous teeth take hold of the string and detach a small bolus, which is swallowed into the buccal mass by rapid thrusts of the odontophore. Around the mantle margin as a whole there is a region of strong ciliation. Here, to a smaller extent, particles may be carried directly across the rim and into the mantle cavity. Some particles too may alight upon the horseshoe-shaped anterior lip, where they are caught up by cilia and travel direct to the mouth. Others again, being rejected, find their way on to the ciliary tracts of the under surfaces of the wings and are carried outwards and cast off. These may include larger and looser particles breaking away from the pallial string.

In addition to the collection of food particles in the pallial cavity, a significant part is played also by the foot in *L. retroversa*. This, however, would appear to be much less important than is the case in the Cavoliniidae and Cymbuliidae. Particles alighting on the free surface of the median lobe receive a covering of mucus and are carried forward by cilia in small strings (P FS) directly to the mouth, along the channel between the lateral lobes. Especially strongly ciliated are the brown-pigmented lateral lobes. These have long, close-set cilia keeping up a swift current carrying further particles, embedded in mucus, to the mouth.

On the ciliated bases or 'Wimperfeld' of the wings, the food-collecting action seems much less important. The cilia are shorter than on the lateral lobes of the foot, the direction of current less decisive. So far as could be determined the cilia of the 'Wimperfeld' beat outwards, away from the foot, and their action on particles alighting on the wings is probably rejectory. The flapping of the wings in swimming also serves to dislodge particles from both the upper and lower surfaces; and it would not appear that particles collected on the wings could be easily transferred across the side lobes of the foot in the direction of the oral funnel. The dorsal surfaces of the bases of the wings, which lie closer against the shell, also possess rejectory currents carrying particles away from the mid-line.

A rejectory action seems also to be carried out by the short triangular lobe or 'balancer' (BAL), which Pelseneer has noticed as an outgrowth of the pallial skirt on the right side. Rather strong ciliary currents here proceed from the edge of the mantle, along the lobe to its tip.

DIGESTIVE SYSTEM

The regions of the gut were described briefly by Pelseneer and Meisenheimer. There is a tiny buccal bulb with an odontophore bearing the radula and a small chitinous jaw. The radula is figured for *L. retroversa* (Fig. 3, left side). Tesch has already illustrated it (1946) for the larger *helicoides*, and the small differences apparent are due chiefly to variations in the inclination of the teeth in the preparations obtained. As stated by Pelseneer (1888) and by Tesch, *Limacina* possesses three teeth in each row, a single-cusped median, flanked by two laterals. The oesophagus leads backwards as a fine tube, lined with short cilia, beating rapidly backwards along its whole length. The salivary glands are minute, secreting mucus through the ciliated roof of the buccal bulb. The narrow part of the oesophagus widens greatly at its posterior end into a conical gizzard, broadest behind, and strongly invested with circular muscle. This organ differs somewhat in shape from that of *Cymbulia*, described by Howells (1936), but is so essentially similar, both in its histology and the arrangement of its teeth, as to need little further description. Each tooth forms a stout thickening of the cuticular lining of the gizzard, terminating in a backwardly curved claw. Four teeth fit very closely together, forming a strong mill filling the whole of the central part of the gizzard. A fifth tooth lies posteriorly, near the threshold where the stomach proper begins; it is smaller and less elevated, forming a flat, thickened sheet of cuticle.

The gizzard, as in all the tectibranchs, is oesophageal, and the stomach, properly so-called, opens widely from it behind. It forms a compact, thin-walled bag, finely ciliated throughout, and serves essentially as an annexe to the single digestive diverticulum. The stomach is illustrated in transverse section in Fig. 5, upper, from which it will be seen to have two exits, the digestive diverticulum and the narrower intestine. In addition, there is a small, more strongly ciliated pocket opening into the stomach opposite the digestive diverticulum. This corresponds, from its structural relations and the nature of its epithelium, to the style caecum which has been found to survive in each of the thecosomatous pteropods investigated. In *Cymbulia* it is a longer, blunt-tipped tube; in *Limacina* it is little more than a rounded pocket only 25 μ in greatest diameter. The epithelium is extremely low, cuboidal or even squamous, 1–2 μ in height, never glandular, and the cilia are long and slender, about twice the length of the cells and always difficult to activate in living material. The contents of the sac, after fixation in Bouin's, consist of a reticulum of mucus, rather denser than in the rest of the stomach. There was never a rotating rod visible in living animals; but from its characteristic ciliated lining, and from the presence in *Cymbulia* of a transparent 'style', it is hard to deny this sac, vestigial though it is, the character of a style caecum. Its function must be little if any at all; the mode of action of the stomach and gizzard is one in which the gentle rotation of a style can play no part. The

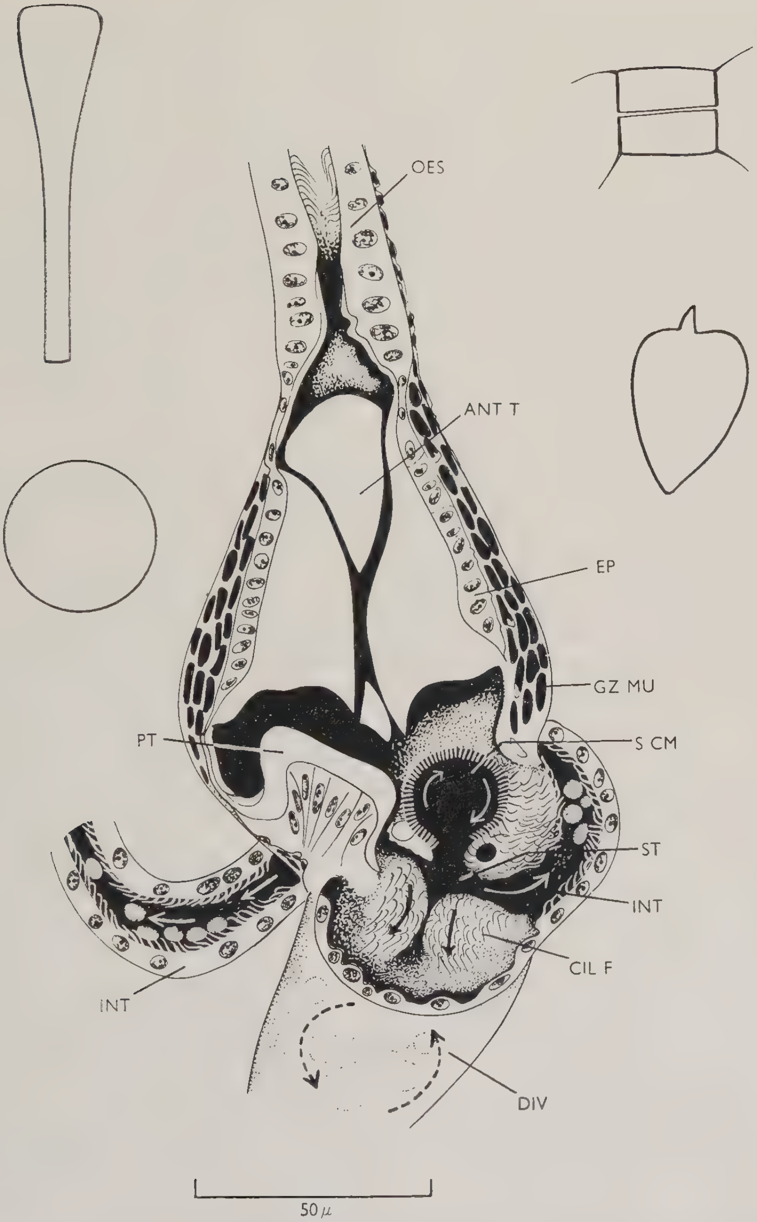

Fig. 4. *L. retroversa.* The gizzard and stomach represented in longitudinal section, chiefly as seen by transparency in the living animal, with structural details supplied from histological observations. The arrows show the directions of the principal ciliary currents. To the same scale are drawn outlines of three species of diatoms and one dinoflagellate, presumed to form part of the food supply of *L. retroversa* at Plymouth. (Left upper, *Asterionella japonica*; left lower, *Skeletonema mediterraneum*; right upper, *Chaetoceros constrictus*; right lower, *Prorocentrum micans*.) ANT T, one of the four anterior teeth of the gizzard; CIL F, ciliated fold at the entrance to the digestive diverticulum; DIV, digestive diverticulum; EP, epithelium underlying a gizzard tooth; GZ MU, circular muscles of the gizzard; INT, intestine; OES, oesophagus; PT, posterior tooth of gizzard; S CM, style caecum; ST, cavity of stomach.

digestive diverticulum is bounded by a pair of rounded folds, closely approximated and with cilia beating outwards from the stomach. Into the intestine, a uniform coat of cilia beats in an anal direction.

The gizzard is a structure perhaps unexpected in a ciliary and mucous feeder, but when looked at in action in the living animal, its presence is less difficult to explain. Its circular muscles are strongly contractile, and its four anterior teeth closely interlock across the lumen. They open apart when the gizzard is distended, to form a most effective mill, through which particles with a diameter of up to 40–50 μ are able to pass. Expansion of the gizzard serves to draw between the teeth a small amount of mucus-bound food from the oesophagus. On contraction hard material is crushed and the contents released in the form of tiny fragments easily able to be received into the mouth of the digestive diverticulum.

Although diatoms could not be fed to *Limacina retroversa* in the laboratory, it is a fair assumption that this species, like the other members of the genus investigated, takes most of its food in the form of diatoms and other phytoplankton organisms, many with hard tests or frustules. For *L. helicoides*, one of the larger sized antarctic species, Dr T. J. Hart has made the following analysis of stomach contents which he has very kindly allowed me to quote: *Fragilariopsis antarctica* (size *c.* 30 μ), *Thalassiosira* (*c.* 35 μ), together with fragments of the larger *Coscinodiscus* and *Chaetoceros*, the latter being very large and dominant, forming 75% of the diatoms at the station referred to (St. 500, South Georgia Survey, 21 November 1930). Larger individuals of *Chaetoceros* spp. have long bristles, reaching 1000 μ in length, and it is obvious that this diatom, together with intact *Coscinodiscus*, was being rejected as unwieldy. Few dinoflagellates were recorded at this station, and no specimens are mentioned from the stomach of *Limacina*. For *L. retroversa* at Plymouth, the figure of the gizzard (Fig. 4) is accompanied by outlines to similar scale of three of the diatoms, *Asterionella japonica*, *Skeletonema mediterraneum* and *Chaetoceros constrictus*, and one peridinian, *Prorocentrum micans*, stated by Lebour (1918) to be especially abundant or dominant in the plankton at the mouth of the Sound, and at E 1, in July and August. The oesophagus of *retroversa*, with its five or six longitudinal folds can be widely distended with the passage of larger particles, and there is no reason to believe any of the above phytoplankton organisms would be found unmanageable. In addition, no doubt, *Limacina* is able to exploit the large resources of nanoplankton, including naked flagellates, though the presence of these quickly digestible organisms in the gut is much less easy to establish.

The passage of carmine particles through the alimentary canal is relatively swift. Material administered in the laboratory and ingested from the pallial food string is received from the stomach into the intestine in 30 min. after ingestion. Carmine is never admitted to the digestive diverticulum, and having once reached the intestine it is discharged from the anus about 2 min.

later, being impelled through the intestine in a loose faecal rope, with the help of both cilia and peristalsis.

The muscular action of the gizzard in *Limacina* provides also a pumping action. There is a constant ebb and flow between the stomach and the digestive diverticulum, of a watery mucoid substance, containing suspended yellowish green droplets as well as black and dark brown particles extruded from the excretory cells of the digestive gland. This material is thoroughly circulated within the stomach, while the intestine is held closed, either by the approximation of its walls, or the presence of more solid faecal particles. At intervals, most of the particles from the digestive gland are forcibly ejected as a compact mass into the intestine. Fragments of diatom cases seldom appear to enter the diverticulum, but find their way straight through to the intestine. There is little muscular contraction by the wall of the stomach proper—most of the compression of the stomach is supplied from the gizzard. Neither are there any evident gland cells in any part of the oesophagus, or stomach. Mucus is provided from the mantle cavity and foot, and in smaller part probably from the salivary glands.

The digestive gland is a long, wide tube forming most of the visceral mass, anteriorly to the gonad. It is dark greenish yellow in colour, packed with particles which appear strongly refractile in the intact animal. The lining of the gland has no cilia—there is merely a tapered flange of ciliated cells from the stomach, running backward into the diverticulum, and soon giving way to digestive tissue.

The structure of the epithelium (Fig. 5, lower) is notable for its strong resemblance to that of *Cymbulia* as described by Howells (1936). The lumen is pocketed into small shallow lobules (50 μ across), set close together by their basement membranes, and lined with cells of three types. The most numerous are the digestive cells, which, at the phase of activity at which they were examined, tend to be squarish or broader than long. They contain two sorts of vacuoles, the basal ones filled with finely granular material staining blue in azan, the more superficial ones on the whole larger, and colourless. The digestive cell at its intact stage has the finely striated border referred to by the present writer (in the press) in the Ellobiidae, and there is throughout a general resemblance between the digestive cells in primitive pulmonates and opisthobranchs. No stages in the formation of 'fragmentation phagocytes' were observed in material of *Limacina*, and from the stage of activity at which the gland was sectioned these are probably not to be expected. The remaining two types of cell are to be regarded as excretory, and both have already been observed by Howells in *Cymbulia*. The first have large rounded nuclei, with prominent nucleoli and chromatin strands and their cytoplasm stains uniformly dark brown with azan or black with Heidenhain's haematoxylin. The second are rather smaller, very broad-based and triangular; their cytoplasm is filled with small distinct spherules staining deeply black with haematoxylin.

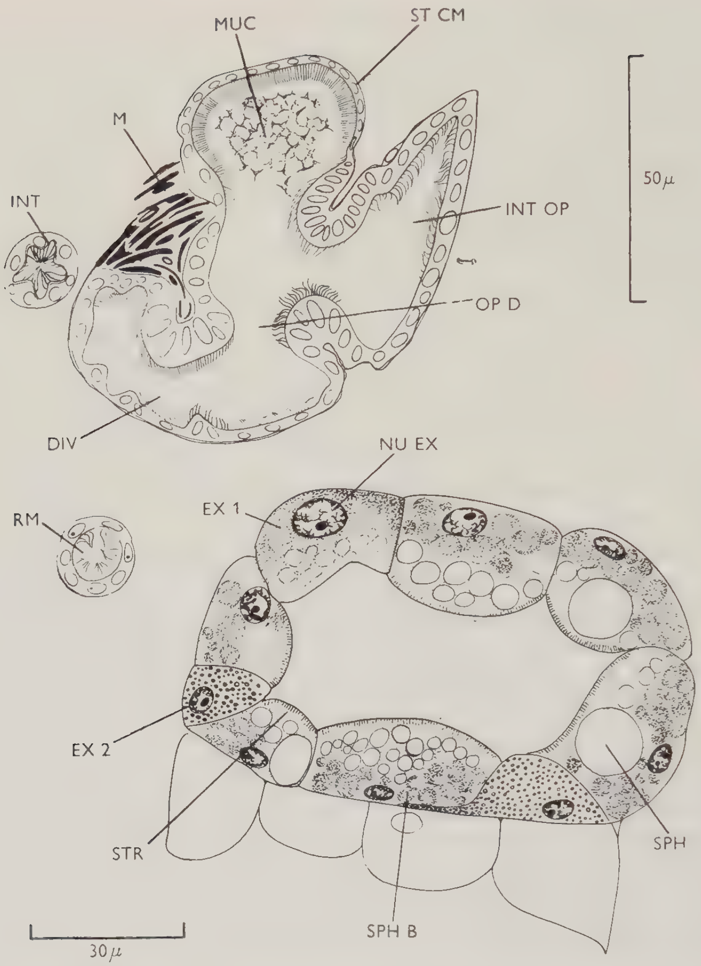

Fig. 5. *L. retroversa.* Upper. Transverse section passing through the stomach, intestine and rectum. DIV, digestive diverticulum; INT, intestine; INT OP, opening of the intestine from the stomach; M, hindmost part of gizzard muscles; MUC, mucous contents of style caecum; OP D, opening of digestive diverticulum from the stomach; RM, rectum; ST CM, style caecum. Lower. Section of a single lobule of the digestive gland. (Bouin's fluid; Heidenhain's azan.) EX 1, EX 2, two types of excretory cells; NU EX, nucleus of excretory cell; SPH, clear spherule of digestive cell; SPH B, blue staining vacuole of digestive cell; STR, striated border of digestive cell.

DISCUSSION

Pelseneer first provided a reliable anatomical account of the pteropods, and he settled the distinctness of the two groups Thecosomata and Gymnosomata. The collective name 'pteropods' survives now only as a convenient term of common reference for pelagic tectibranchs. Thus the more recent classification adopted by Thiele (1931) and by Hoffmann (1939), reverting to a single order Pteropoda as a major division of the Opisthobranchiata, must be regarded as an altogether retrograde step. The Thecosomata and Gymnosomata are unlike in almost all features with the single exception of their habit of swimming. They are almost certainly separately derived from bottom-dwelling groups of tectibranchs. The Thecosomata are entirely ciliary feeding; the Gymnosomata are active carnivores, very often feeding on small thecasomes, and in the majority of their characters they are very much more specialized. It is hoped in a forthcoming paper to give a better account of the anatomy and reproductive history of the British gymnosome *Clione limacina.*

Our knowledge of small thecosomatous pteropods is rather better than for the Gymnosomata. Here we have the advantage of an animal with a hard shell, or a pseudoconcha, which suffers much less damage in collecting and survives longer in the laboratory. Of this group we now have an adequate knowledge of the feeding mechanism, digestive system, sexual succession and reproductive organs; and in oceanographic literature *Limacina* is well known for its ecological importance as a grazer on phytoplankton. All our evidence confirms Pelseneer's derivation of these animals from bullomorph tectibranchs; an instructive parallel is provided by *Acera* which has developed an ability to swim by the enlargement of its parapodial lobes, though this animal has probably little to do with the actual phylogeny of the pteropods. *Limacina*, which is still shelled, spirally coiled and operculate, is in all ways an excellent form for an understanding of the transition to pelagic pteropoda.

Pteropods have been previously looked at as living animals chiefly by Yonge (1926). He did not, however, examine *Limacina*, and in this generalized form there are now several differences to report from the Cavoliniidae and Cymbuliidae. Paradoxically, so small a creature as *Limacina* is much easier to examine intact and living than the larger pteropods; its mode of feeding and the action of the gut are readily observed through the transparent shell, and it remains happily alive in sea water in the laboratory for many days.

It is admitted by all that the three families of Thecosomata, the Limacinidae, Cavoliniidae and Cymbuliidae, stand in that order of specialization; and within the Cymbuliidae it is probable that *Gleba*, as suggested by Yonge, is the most advanced genus. In food-collecting the greatest point of difference is the absence in the Cavoliniidae and Cymbuliidae, as reported on by Yonge, of any trace of pallial food-collecting with the passage forward of a mucous string. In *Cavolinia* and *Creseis* the whole of the food-collecting is done by the 'Wimperfeld' of the wings and by the side lobes and median lobe of the foot. In *Cymbulia* the ciliary action of the 'Wimperfeld' is lost, and the emphasis is on feeding by the foot. Food particles travel to the mouth along two obliquely transverse grooves, bounded in front by the two lateral lobes, forming raised margins, and behind by the unpaired median lobe. The mouth is depressed into a funnel into which these ciliary currents lead downwards. The ciliary tracts on the wings are much reduced and there is little if any collection of food; the flapping of the wing dislodges alighted particles. In *Gleba* there is the same situation in essentials, but the parts of the foot are here drawn out into a long, spatuliform 'proboscis', lying parallel to the surface of the wings, with the mouth in a central cleft near the tip. Ciliated grooves carrying food particles proceed along the rounded edges of the 'proboscis', the lateral lobes of the foot forming the upper margin and the highly modified median lobe the lower.

A glance at Pelseneer's figures for *Cavolinia* and *Cymbulia* shows why pallial food-collecting cannot take place in these forms, at least by a food string emerging anteriorly to the head in the method used by *Limacina*. In these higher pteropods the visceral mass has turned through 180° to bring the anus to the left side of the mantle cavity of the now bilaterally symmetrical body. The position of the mantle cavity is now ventral, and its opening is separated from the mouth by the interposition of the foot and wings. In the floating and swimming position, especially in *Cymbulia*, which lies on its dorsal surface with the ventral mouth and foot uppermost, this change in orientation of the mantle cavity may be explained by reference to the requirements of the animal in flotation.

A food string from the mantle cavity would thus be unable to pass to the mouth in the same way as in *Limacina*, and it is difficult to visualize how *Cavolinia* or *Cymbulia* might make use of the mantle in feeding. But the problem remains of the relatively huge pallial gland developed in cavoliniids and cymbuliids just as in limacinids. In the latter its function is evidently wholly concerned with feeding, and its survival in the higher families is puzzling to explain in the absence of a mucous feeding role. From the histology of the pallial gland and from the evidence of simplification of the pallial complex, this gland is almost certainly not a direct survival of the hypobranchial gland of prosobranchs which is a different structure that was lost with the gill. The function of the hypobranchial gland—detritus removal and sanitation of the pallial cavity—is scarcely in the least likely to be important in a pelagic mollusc in clear water. Further, the histology of the pallial gland is identical in all the Thecosomata, and we are clearly dealing with the same structure in each of the three families. While the issue of a mucous string from the pallial cavity in Cavoliniidae is feasible, with a changed course along the foot, it is not easily possible in Cymbuliidae which have a wide spread of the wings almost entirely closing off the opening of the pallial cavity. It may yet be worth further investigation of these pteropods to see if the pallial cavity and its gland actually play any role in collecting food.

Whatever the role of the pallial cavity in the Cymbuliidae, the ciliary collecting fields on the wings are greatly reduced, and in *Gleba* they appear to be quite lost. In the Cavoliniidae, where they are probably best developed, they are even here of much less importance than the lobes of the foot. In the Limacinidae they seem to be of minimal importance in feeding; more than any other family this group retains the use of the widely expanded median lobe or 'sole' of the foot.

Of other evolutionary trends connected with feeding in Thecosomata, we may mention the reduction of the buccal mass. The Cavoliniidae have this structure moderately well developed, with salivary glands, jaws and radula. The same is true of the less advanced Limacinidae. In *Cymbulia*, Yonge states that the buccal mass is 'much less developed than in the two species already described', and in *Gleba* there is 'no buccal mass, hence no jaws, radula or salivary glands'. It is lacking also in *Corolla*.

The essential structure of the stomach and gizzard is fairly constant in all these groups. The gizzard was regarded by Yonge as probably another structure 'handed down from carnivorous ancestors and clearly of little use to an animal which feeds by ciliary mechanisms'. In *Limacina*, however, the gizzard has only to be seen in action to realize its usefulness, both as a diatom mill and, by the action of its muscular wall, as a pumping device, regulating the inflow and outflow from the digestive diverticulum. The important part played by muscle, even in a ciliary feeder, is well emphasized in a creature small enough to view with its working stomach intact, and, as stressed by Yonge (1949) in connexion with the eulamellibranch Tellinacea, we should not undervalue the role of muscle in the digestive economy of ciliary feeders. Comparison may be made with *Scaphander* which uses its gizzard to crush the shells of small bivalves, and here differs only in its size and choice of food from *Limacina*.

The tiny style sac of *Limacina*, though of much smaller size, corresponds exactly to that described by Yonge in *Creseis* and by Howells (1936) in *Cymbulia*, and said by Meisenheimer (1905) to be retained in all Thecosomata. Here it can be little more than a vestige, as it is impossible to see of what use a style could be in its normal role of stirring and moving the stomach contents. Moreover, an amylolytic enzyme has never been demonstrated in Thecosomata, and from the nature of the food is likely to be little needed. The survival of the style sac in this group once again impresses us with the great unity in the pattern of the stomach shown among so many of the mollusca.

SUMMARY

The biology of the thecosomatous pteropod, *Limacina retroversa*, is discussed with special reference to the swimming mechanism, mode of food collection and structure and action of the alimentary canal. The ciliary currents of the foot and pallial cavity are described in detail, in relation to the collection of food particles, and it is shown that the pallial cavity, with its mucus-secreting pallial gland, plays the principal role in gathering food from the surrounding water. A mucous food string is compacted and carried forward to the mouth.

The structure of the gizzard and the stomach is described, and the toothed, muscular gizzard is shown to form a mechanism for crushing diatom frustules. The vestigial style sac, and the epithelium of the digestive diverticulum are described and compared with those of other Thecosomata.

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NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

III. A COMPARISON OF *DIDEMNUM MACULOSUM*, *D. CANDIDUM*, *D. HELGOLANDICUM*, AND *TRIDIDEMNUM ALLENI*

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(Text-figs. 1–5)

When Michaelsen (1923) described a species of didemnid from Heligoland, he stated that it was very similar to *Leptoclinum maculosum* Milne Edwards (1841), so far as may be judged by external appearance, but since no adequate description of this latter existed he proposed to call his Heligoland form by a new name, *Didemnum helgolandicum*, rather than use an old name which might well be wrong. Neither Michaelsen nor Hartmeyer, who described further details of *D. helgolandicum* (1924), was able to compare it directly with specimens of *D. maculosum* from the Channel coasts whence it was first described by Milne Edwards. Through the kindness of the directors of the Zoological Museum of Copenhagen and of the Biological Station of Roscoff I have been able to obtain specimens of both these forms and to compare them with specimens collected around Plymouth and on the coast of North Wales, and with specimens of *D. candidum* Savigny, collected at Naples, and from the Red Sea, and of *Trididemnum alleni* Berrill, collected around Plymouth.

MATERIAL EXAMINED

Three small colonies of *Didemnum helgolandicum* collected by Ørsted in 10–13 fathoms 5 'quarter-miles'[1] north-west of Hirtshals (one of Michaelsen's type localities) and determined by Michaelsen. This is part of the type material.

About twenty colonies of *D. maculosum* collected at Roscoff (one of Milne Edwards's type localities) in the *Laminaria* zone, and one specimen from the same locality determined by Pizon.

Six colonies of *Didemnum maculosum* from various points on the North Wales coast.

About 200 colonies of *D. maculosum* and 100 of *Trididemnum alleni* from the Plymouth area (Berrill's type locality) collected and determined by Miss P. Kott, and in the local collection of the Plymouth Laboratory.

About 600 colonies of *Didemnum* spp. from the Plymouth area collected by myself, the identification of which is discussed below, and about 300 colonies of *Trididemnum alleni*.

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[1] If these are Danish miles, since Ørsted collected the specimen, this distance is *c.* 8·5 km.; if, however, they are German miles, since Michaelsen wrote the present label, then the distance is *c.* 9·4 km.

Twelve colonies of *Didemnum* sp. collected by me from the Secca di Benda Palummo in 40 m. and from other localities at Naples.

Two colonies of *D. candidum* in the local collection of the Naples Zoological Station determined by Traustedt.

One specimen of *D. candidum* from the Red Sea (Savigny's type locality) determined by Garstang.

Nine specimens of *D. candidum* from the Red Sea, four colonies from the Suez Canal, and thirteen colonies from the Great Barrier Reef (Australia), in the British Museum collection.

OBSERVATIONS

When examining specimens of '*D. maculosum*' collected in the Plymouth area I soon realized that I was dealing with two separate species. The difference was most obvious in the larvae, one of which was twice as long as the other. An examination of the specimens in the local collection of the Plymouth Laboratory showed that these too fell into two groups, though unfortunately none of the specimens of one group had any larvae. The larvae and adults of the other group of specimens agreed in general appearance with those described under the name of *D. maculosum* auctt. by Millar (1949).

Here, then, were two species under the name *D. maculosum*. One of them I have identified with *D. candidum* Savigny, the other with *D. helgolandicum* Michaelsen. About one-quarter of the specimens collected in the Plymouth area agreed with Michaelsen's description of *D. helgolandicum* and with the specimens identified by him which I have had the opportunity to examine. All the other specimens from Plymouth, those from North Wales, those from Roscoff, and those from Naples, agreed with Savigny's (1816), van Name's (1945) and Michaelsen's (1920) descriptions of *D. candidum* and with the specimens of this species which I have examined. These latter specimens, especially those from Roscoff, are presumably the *Leptoclinum maculosum* of Milne Edwards, so this name must be abandoned in favour of the older name *Didemnum candidum* Savigny (1816), the type species of the genus. *D. helgolandicum* is different from this, as Michaelsen well knew, for he had the opportunity of examining specimens from near Savigny's type locality (Michaelsen, 1920). Accordingly, Michaelsen's name stands since it is not a synonym of any earlier described species. It is fortunate that the name to be abandoned is that of Milne Edwards with its insufficient description.

The descriptions which follow are based primarily upon the Plymouth material.

The colonies of the two species are indistinguishable externally. Both are thin encrusting forms, 1–2 mm. thick, growing on *Laminaria* holdfasts, gorgonians, other ascidians, in fact on any organic substratum and even occasionally on rock. The spicules vary in abundance, but usually it is possible to see the zooids or at least their position. The colour is very variable, whitish, blue-violet, violet, grey, brownish grey, where there is much mud, white, yellow ochre or orange in cleaner situations. The larger colonies are usually marked with lines of deeper colour, which are usually furrows running over the surface in an irregular reticulum. The deeper colour seems to be a result of the less density of spicules in these furrows. The pigment appears to be free in the test, and is not contained in special pigment cells. It rapidly fades in alcohol, more slowly in formalin. The surface of the colony of each species is marked with small granulations most commonly, though they may be missing; these are wart-like protrusions, one near each branchial opening. There may be a layer of bladder cells at one or both of the surfaces of the colony, but this is not constant in either species.

The spicules are most abundant in the upper layers of the colony, immediately below the layer of bladder cells if these are present, and especially clustered in the wart-like protrusions and around the buccal siphons. They diminish in abundance steadily towards the deeper layers, but in *D. helgolandicum* there is frequently a greater abundance in the basal layer itself. In *D. candidum* there is rarely any special density near the zooids, but in *D. helgolandicum* the test immediately surrounding the zooids and especially that part under the abdomen of each zooid is denser in spicules than the rest of the test. The stippling in Fig. 1 gives an indication of the relative density of spicules in the various areas of the test.

The spicules are rather similar in general form, but rather more variable in *D. candidum*. In this species the rays most commonly have a terminal angle of about 45°, but they may be rounded, almost square-tipped, or even rod-like. The most common formula (see Carlisle, 1953) is 1, 6, 9, but 1, 6, 12, 1, 4, 8, or even 1, 6, 12, 24 may occur. The variability in spicule form is greater at Plymouth than is illustrated by Van Name (1921) for West Indian colonies. In *D. helgolandicum* the most common terminal angle for spicule rays is 60° and variations from this form are few. The formula is most frequently 1, 4, 8, 14, but 1, 6, 12, 1, 4, 8 and 4, 8 occur, or even 1, 4 in the lateral thoracic organs, where smaller spicules tend to accumulate, and around the lobes of the buccal siphon. In both species the size range is 25–45 μ with a few smaller or larger (absolute limits: 4 and 75 μ). Those of *D. candidum* tend to be on the average rather larger than those of *D. helgolandicum*.

In *D. candidum* the zooids are rarely arranged in definite systems except around the edges of young colonies. Where a system is distinguishable the zooids are arranged around a common cloacal aperture with the atrial apertures facing inwards. The ring of zooids thus presents an outer aspect (outer with respect to the ring) of ventral surfaces. In *D. helgolandicum*, in the Plymouth material especially, though also in the material from Hirtshals, systems of zooids are much more definite and more commonly developed. Here, however, they are arranged the other way round, with the atrial apertures turned outwards from the ring of zooids and the ventral sides inwards. The centre of such a ring of zooids is solid test; the common cloacal system runs between the systems of zooids. I have never seen this type of system developed in any other species of ascidian.

The zooids of both species are arranged nearer to the vertical than is usual in didemnids. They are never quite vertical, and the abdomen is more inclined than the thorax, so that the neck is bent, especially in *D. candidum*. The total length of a zooid of *D. candidum* in the usual contracted condition of preservation is about 1·1 mm.; in the expanded condition it may measure up to 1·6 mm. The corresponding figures for *D. helgolandicum* are 0·9 and 1·3 mm. The difference is chiefly a result of the longer neck and slightly larger abdomen of *D. candidum* (see Fig. 2).

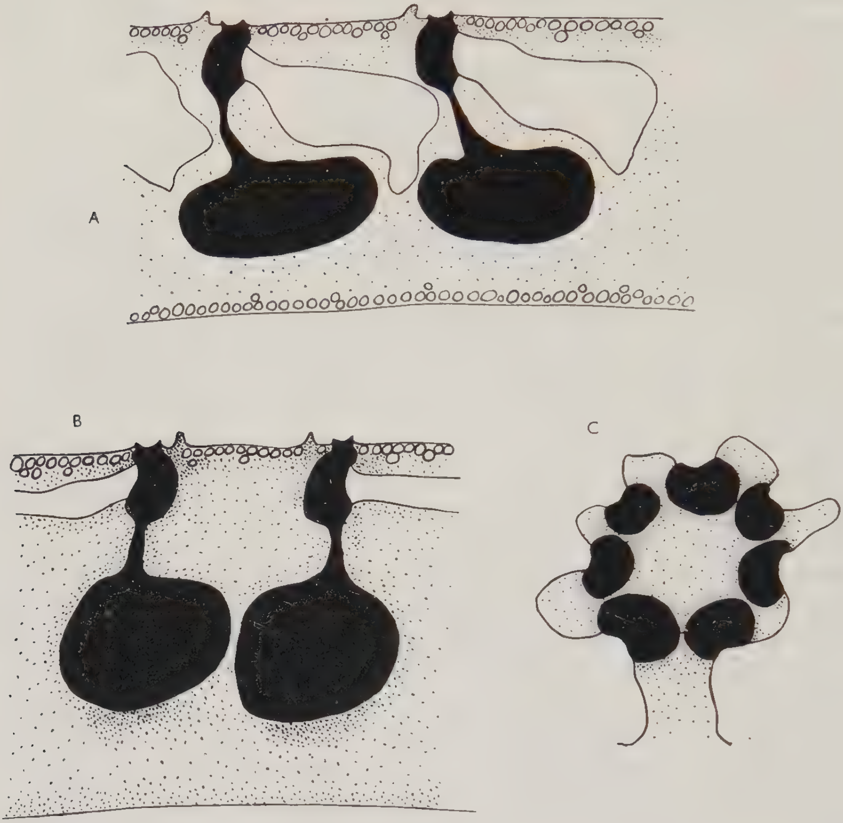


Fig. 1. Sections through colonies of *Didemnum candidum* and *D. helgolandicum*. A, vertical section through part of a colony of *D. candidum*; B, vertical; and C, horizontal section at the thoracic level, through part of a colony of *D. helgolandicum*. The stippling indicates the relative abundance of spicules in the various parts of the colony. Notice the association of the zooids of *D. helgolandicum* into systems with the ventral sides adposed.

The thorax of both species is smaller than the abdomen. There is a cline in *D. candidum* in this respect; the difference in size between the two parts, in European waters, is more pronounced farther north. It is most pronounced on the coast of North Wales, less so at Plymouth, still less at Roscoff, less again at Naples, while in the Red Sea the thorax is little smaller than the abdomen. This may be, at least partly, a result of temperature or nutrition differences. The difference is least marked in older, larger zooids, and colonies grown in warmer waters grow faster to a given size so that there is a less proportion of small zooids. This is not, however, the whole explanation for differences in proportion exist even between zooids of the same size from the different localities. The thorax is separated from the abdomen by a long neck, which in *D. candidum* is about the same length as the thorax, sometimes

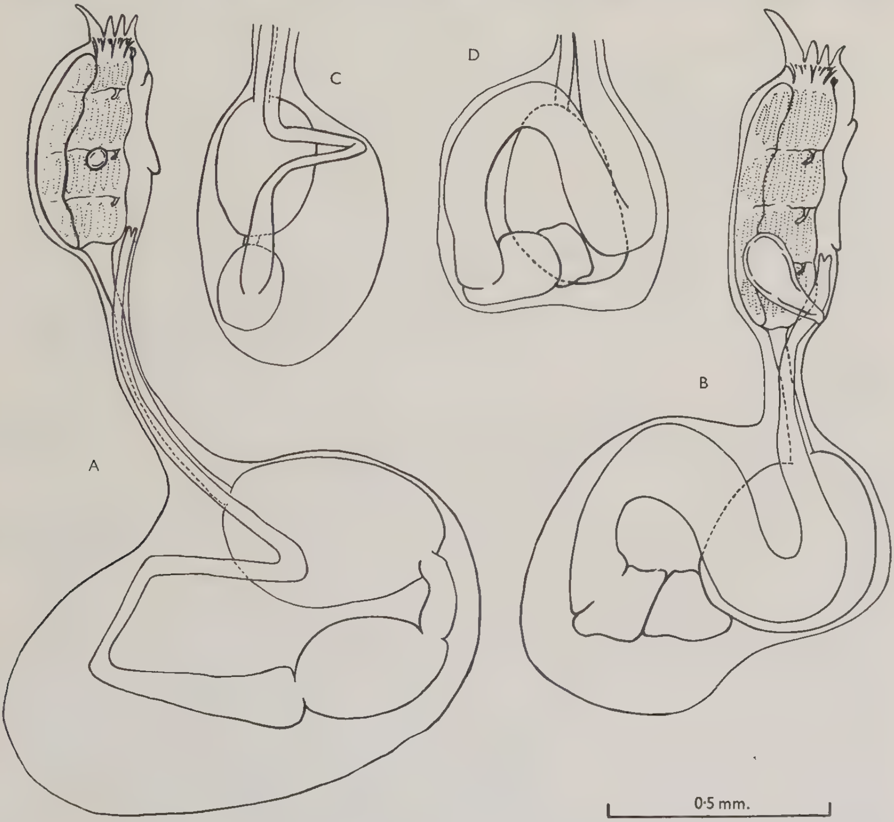


Fig. 2. Drawings from the left sides of zooids of (A) *Didemnum candidum*, and (B) *D. helgolandicum*; and from the ventral of sides of abdomina of (C) *D. candidum*, and (D) *D. helgolandicum*. The entire genital system and pyloric gland are omitted. The buccal lobes and tentacles are drawn only from one side, omitting those of the other.

shorter, sometimes longer, while in *D. helgolandicum* it is always shorter than the thorax, usually less than half. The relative measurements refer especially to the uncontracted condition; the neck suffers more from shrinkage than most other parts of the body, and in a contracted preserved condition it is frequently much shorter.

The branchial siphons are six-lobed in both species though occasionally an eight-lobed individual of *D. candidum* may be found. The atrial aperture is not prolonged into a siphon and does not possess a languet. It remains as a simple opening which is round in the expanded animal but contracts to a slit. This slit is transverse and crescentic in *D. helgolandicum* and convex anteriorly, while in *D. candidum* it is usually longitudinal.

The lateral thoracic organ of *D. candidum* is small and placed a little behind the middle of the thorax. It is about half the height of a row of stigmata. In *D. helgolandicum* the lateral thoracic organ is rather larger. It is placed level with the third interstigmatic transverse bar and is about the height of a row of stigmata. In some colonies it departs from the usual circular or oval shape common in didemnids by sending out a dorsally directed extension, so that the organs on opposite sides of the thorax are often united into a saddle-shaped mass, like the thyroid body of mammals. The rim is raised rather more in this latter species.

Both species show sixteen buccal tentacles in a well-grown zooid. These are of four or possibly only three orders of size arranged 1, 4, 3, 4, 2, 4, 3, 4, 1 or 1, 3, 2, 3, 1, 3, 2, 3, 1. Smaller zooids have only eight tentacles.

The majority of zooids of both species have four rows of stigmata, but zooids may be found in most colonies with three or five rows (see also Carlisle, 1954). In *D. candidum* there are usually six or seven stigmata in a half-row; in *D. helgolandicum* eight or nine.

A thoracic retractor process is frequently developed in zooids of both species. It is usually situated to the right of the thorax; it is never attached to the neck.

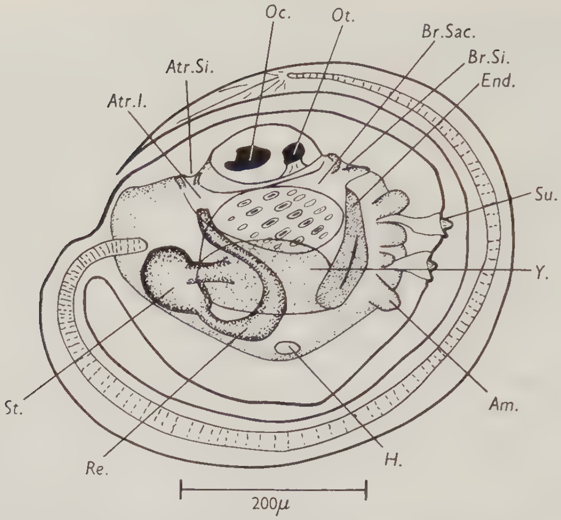
The oesophagus of *D. candidum* is about as long as the thorax; in *D. helgolandicum* it is about half the length of the thorax. In both species it is more or less straight and opens into a globular stomach which may be rather strongly laterally compressed in *D. helgolandicum*. The post-stomach of *D. candidum* leaves the posterior end of the stomach and runs vertically to the globular mid-intestine, which is the most posterior part of the gut-loop and is sometimes nearly half as large as the stomach though usually no more than one-quarter. In *D. helgolandicum* the post-stomach leaves the ventral side of the stomach and runs horizontally; it is the most posterior part of the gut-loop. It is short and thick and opens into a short, thick mid-intestine which is much smaller than the stomach. From the mid-intestine the rectum, in *D. candidum*, runs ventrally with an enlarged proximal portion, then takes an abrupt turn anteriorly; another abrupt turn to the left produces an elbow which sticks out sharply to the left of the stomach before the rectum comes parallel to the oesophagus to run up the narrow neck. The anus in this species is level with the middle of the last row of stigmata. In *D. helgolandicum*, on the other hand, all the curves in the course of the rectum are smooth and gradual. It leaves the mid-intestine, with no proximal swelling, in an anterior direction, curves round in a smooth S-shaped curve, lying partly to the left of the stomach, but without any pronounced elbow, and then runs up the neck beside the oesophagus. The anus is level with the hinder end of the third row of stigmata. The tight S-shaped curve of the rectum of *D. helgolandicum* is reminiscent of that of *Trididemnum niveum* (see Carlisle, 1953).

The epidermal ampullae are short and inconspicuous in both species, but rather variable in development, perhaps depending on the stage of growth.

In *Didemnum candidum* the vas deferens makes between seven and ten turns around the testis, with eight perhaps being the commonest number. Zooids may however be found, even within the one colony, with as many as twelve turns of spire, or as few as six. In *D. helgolandicum* there is similar variation with nine as the most common number of turns of spire. The testis itself in both species is a single spherical vesicle about as big as the stomach. The ovary is of the typical didemnid form with one predominant large ovum at a time and a series of progressively smaller ones.

The size of the larva of both species is somewhat variable: as in most species of didemnids the small peripheral zooids of the colony produce smaller ova and hence smaller larvae than do the larger central zooids. But the larvae from a zooid of *D. helgolandicum* is approximately twice the size of one from an equal-sized zooid of *D. candidum*. The body length, excluding tail, of a larva from a medium-sized zooid of *D. candidum* is approximately 230 μ, that from *D. helgolandicum* 630 μ.

The larva of *D. candidum* is unusual in possessing only two suckers set vertically, one above the other. They are cup-shaped with a conical cement gland set in the centre of each. They are flanked by three or four ampullae on each side. There are three suckers in *D. helgolandicum* and these are flanked by six or seven ampullae on each side. The cement glands in the centres of the suckers are rather more obtuse angled than is usual. The ampullae are relatively rather longer than in *D. candidum*. The endostyle is set more vertically in *D. candidum* than in *D. helgolandicum*, where it is inclined at about 45° to the axis of the larva. The branchial sac, at the moment of hatching, is perforated usually with four rows of stigmata, each of four to six stigmata per half row in *D. candidum*, or six to eight in *D. helgolandicum*. In smaller larvae from the periphery of the colony, however, there may be only three rows; in this case a fourth row does not perforate at least up to metamorphosis beyond which stage I have not observed them further. Below the sac is a mass of yolk-laden cells which persists far into free-swimming life. The gut remains as a solid rod, without lumen, up to metamorphosis. In *D. candidum* it lies in the normal position for the family, with a horizontal oesophagus


Oc.
Ot.
Br.Sac.
Atr.Si.
Br.Si.
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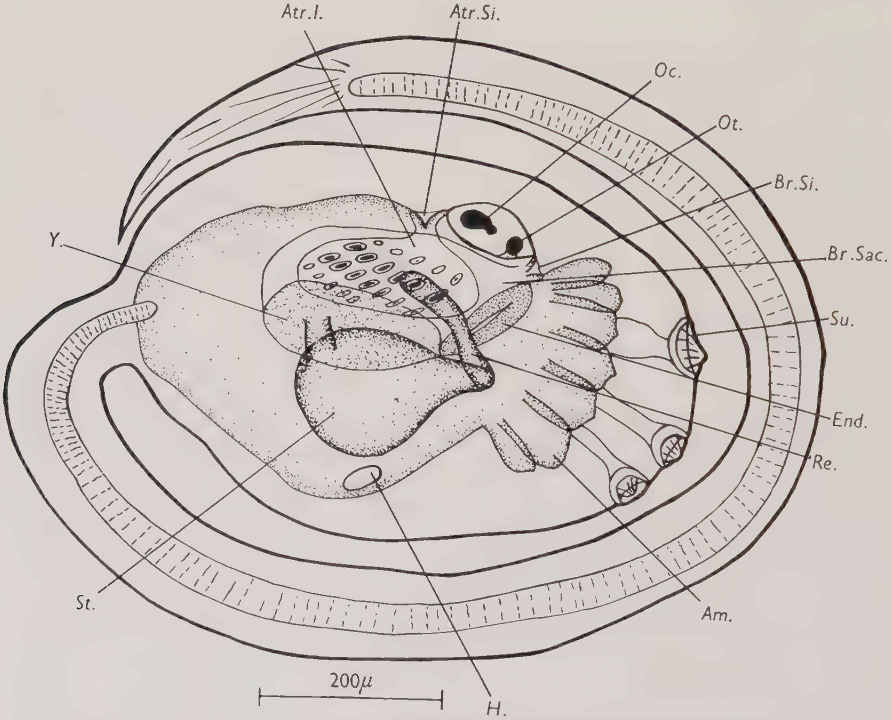
Atr.I.
Su.
Y.
St.
Am.
Re.
H.
200μ

Fig. 3. Larva of *Didemnum candidum* from the right side. *Am.*, ampulla; *Atr.I.*, atrial invagination; *Atr.Si.*, atrial siphon; *Br.Sac*, branchial sac; *Br.Si.*, branchial siphon; *End.*, endostyle; *H.*, heart; *Oc.*, ocellus; *Ot.*, otolith; *Re.*, rectum; *St.*, stomach; *Su.*, sucker; *Y.*, yolk.


Atr.I.
Atr.Si.
Oc.
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Y.
Br.Sac.
Su.
End.
Re.
St.
Am.
200μ
H.

Fig. 4. Larva of *Didemnum helgolandicum* from the right side. Lettering as in Fig. 3.

projecting back from the branchial sac or from the yolk mass, leading to a posteriorly situated (posteriorly with respect to the swimming axis of the larva) globular stomach. The intestine-cum-rectum leaves from the antero-ventral corner of the stomach and sweeps round in a wide S-shaped curve to finish in the atrial cavity above the oesophagus. In *D. helgolandicum* the whole gut is much farther forward than is usual in the family. The oesophagus leaves the branchial sac or yolk mass in a downwards direction and runs into the stomach, which lies below the yolk mass, at its posterior end. The stomach is pyriform with the front end narrower. From this front end, even in front of the endostyle, the intestine-cum-rectum departs at right angles, lying transversely across the body. It then runs up at an angle of 45° almost straight to finish in the atrial cavity. The heart is in the usual position in both species, but in *D. candidum* the gut is entirely behind it, whereas in *D. helgolandicum* it lies beneath the stomach.

The sensory vesicle contains both ocellus and otolith, which are large and well developed in both species. The tail is of the same relative length in both, extending around the body, just before hatching, almost as far as its own base.

The free-swimming period is about 1 hr. in *D. candidum*, 4 hr. in *D. helgolandicum*.

Leptoclinum maculosum Milne Edwards is in some respects different from *Didemnum candidum* Savigny from the type locality, in particular in the relatively larger abdomen and in the swelling of the mid-intestine, but in all other respects they are alike. The 'elbow' which the rectum makes (which can be seen in Savigny's drawing, 1816, pl. XX, fig. 1) occurs in both; this character is quite unlike anything found in other species of *Didemnum*. The larvae of *D. candidum* from the Red Sea share with the specimens of '*D. maculosum* auctt.' the possession of only two suckers, a character which is shared only by *Trididemnum alleni* among ascidians. In fact the larvae from Red Sea specimens and from Plymouth specimens are indistinguishable. If we consider the two characters mentioned above, in which the two forms differ, the relative size of the abdomen and of the mid-intestine, we find that there is a cline in both these characters. Where specimens from Naples differ from those from the Red Sea in these two characters they do not differ enough to justify separate specific rank. Where those from Roscoff differ from those from Naples the difference is again not enough to justify specific separation. Again Roscoff and Plymouth specimens do not differ more than one expects to find within a species, nor do Plymouth and North Wales specimens. But direct comparison of specimens from North Wales with those from the Red Sea would suggest that they might conceivably be different but closely related species, or more likely, geographical subspecies. Since, however, intermediates are provided from the stations in between, I do not propose even subspecific rank for the English forms. I regard them as representatives of the extreme northern end of a cline with a complete range of intermediates down to the type-locality in the

Red Sea. They differ from the Red Sea specimens only in the relatively larger abdomina and in the greater degree of swelling of the mid-intestine. It is to be noted that the Red Sea specimens also show some degree of swelling of the mid-intestine, as may be seen in Savigny's drawing (1816, pl. XX, fig. 1[4]).

T. alleni Berrill (1947) is a form described from Plymouth. It seems to represent dwarf or young stages of *Didemnum candidum*. It has already been noted (above and in Carlisle, 1954) that the marginal zooids of *D. candidum*, and indeed of many species of *Didemnum*, have only three rows of stigmata and a simplified gut. Moreover, such zooids produce smaller larvae with only three rows of stigmata. In fact, the marginal zooids of *D. candidum* are identical with the zooids of *Trididemnum alleni*, and so also are the larvae of such zooids. I would suggest that *T. alleni* is the product of such a dwarf larva (or even occasionally of one of the larger larvae), which has settled in a place where there is either little food or little space for expansion. In such a situation the colony is bound to remain small, the zooids are likely to be smallish, hence the simplified gut, and unlikely to produce the larger branchial sacs which are characteristic of flourishing zooids when they replace a worn-out sac by a newly budded one. Direct observation in the field supports this hypothesis. I have on several occasions observed a scatter of young colonies evidently formed from simultaneously released larvae from one parent. Such a scatter settling around the base of a gorgonian or of a *Cystoseira*, some on the rock and some on the organism, consists of identical colonies. If one or two are removed they are usually found to consist of about six to ten zooids, of which one only may have four rows of stigmata; this doubtless is the oozooid. The other zooids are typical *Trididemnum alleni*. But in the course of 2 or 3 months, the colonies growing on the rock and on the larger expanses of substrate have developed into typical *Didemnum candidum* with four rows of stigmata in all but the marginal zooids. The colonies on restricted space have hardly grown at all and have remained in the *Trididemnum* condition with only very few zooids, if any, possessing four rows of stigmata. Such a colony transplanted to a position where feeding is better and where there is room for expansion soon develops into a typical *Didemnum candidum*. *Trididemnum alleni* is thus only one form of *Didemnum candidum*.

Geographical Distribution

D. candidum is a tropical and temperate species extending from the West Indies to the East Indies, New Zealand and Japan (Fig. 5). It does not apparently occur in the main body of the Pacific, or on the western shores of America. North and south it is bounded more or less exactly by the 15° C. isothere.

D. helgolandicum is only known from Heligoland, the Skagerrak (Michaelsen, 1923), Faeroes (Hartmeyer, 1924) and Plymouth.

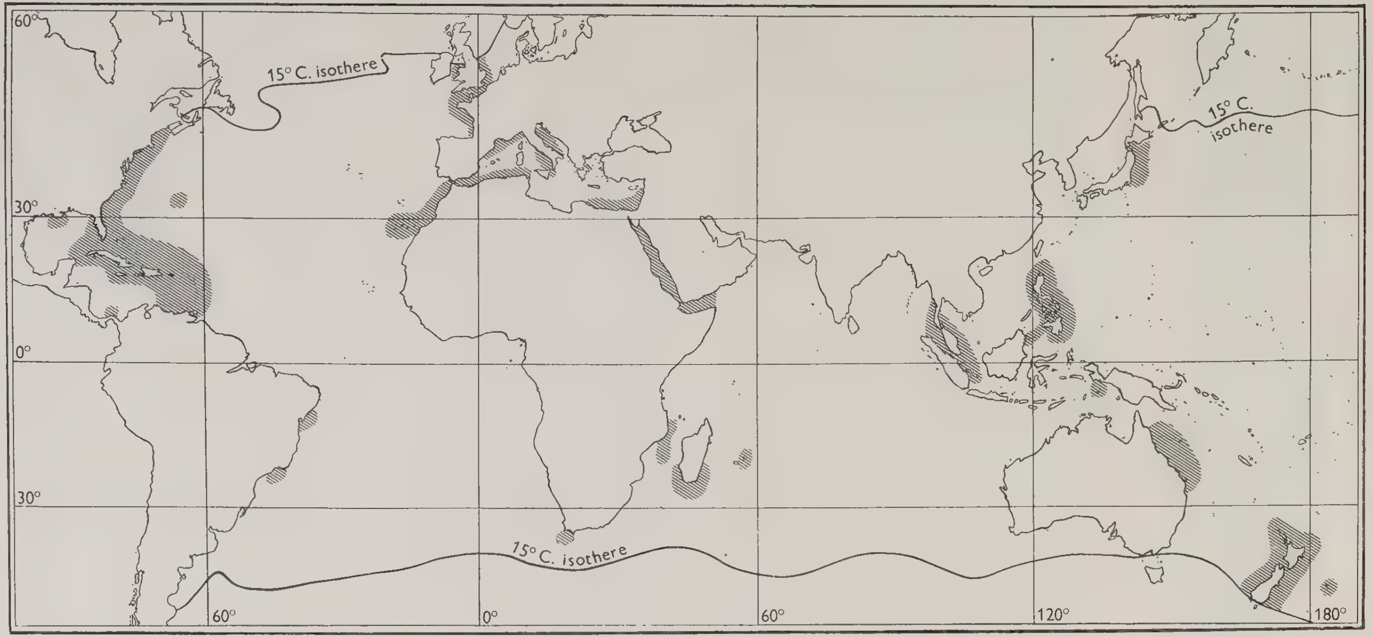
60°
30°
0°
30°
60°
0°
60°
120°
180°
15° C. isothere
15° C. isothere
15° C. isothere

Fig. 5. Map of the distribution of *Didemnum candidum* (including *D. maculosum*, *D. candidum lutarium* and *Trididemnum alleni*).

SUMMARY

'*D. maculosum*' as found at Plymouth is a mixture of *D. candidum* Savigny and *D. helgolandicum* Michaelsen. Material from Roscoff, Naples and the north coast of Wales is all *D. candidum*. *Trididemnum alleni* is one form, a dwarf or young stage, of *Didemnum candidum*. The adults and larvae are described and compared.

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J. Mar. biol. Ass. U.K. (1954) 33, 325–327
Printed in Great Britain

NOTES ON THE DIDEMNIDAE (ASCIDIACEA)

IV. THE INCREASE OF *TRIDIDEMNUM NIVEUM* (GIARD) IN THE PLYMOUTH AREA, AND THE STRUCTURE OF ITS LARVA

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(Text-fig. 1)

Since I first found *Trididemnum niveum* (Giard) at Salcombe in the autumn of 1951 (Carlisle, 1953) this species has spread and increased greatly in the Plymouth area. I have found it in abundance at Salcombe (Castle rocks and the Salstone), Wembury, and especially at Looe around the Island. The favourite habitat at all these places is the same as that at Roscoff, on the fronds of *Cystoseira*. It occurs rather less frequently on the holdfasts of *Laminaria ochroleuca* and occasionally on other species of *Laminaria*, but very little on any other substrate. It is not truly intertidal; the majority of specimens are to be found just below low-water mark of spring tides, and very rarely are any seen above low-water mark of even the lowest tides of the year.

In March 1952 a few specimens of *Trididemnum niveum* were found at each of the localities listed above. By June the numbers were greatly increased and by October still further. The following March (1953) the numbers were about the same as in the previous June, greater, that is, than the previous March by a factor of about three times. By midsummer *T. niveum* was the most abundant didemnid, and possibly the most abundant ascidian, at Looe in the neighbourhood of the island. At Salcombe it lagged behind *T. tenerum* and *Didemnum candidum* in abundance; at Wembury it was between *D. candidum* and *Trididemnum tenerum* in numbers, but was perhaps more conspicuous than either. In September 1953 it was still the most abundant didemnid at Looe, the second most abundant at Wembury and the third at Salcombe. In March 1954 it is by far the most abundant ascidian at Looe, forming at least 75% of the total ascidian fauna at low-water mark.

The association with *Laminaria ochroleuca*, which species was first observed in Britain in the Plymouth area in 1946 (Parke, 1948), and which is still spreading steadily, might suggest perhaps that *Trididemnum niveum* has arrived from Brittany with this oar-weed and is spreading along with it, perhaps at a distance of 2 or 3 years behind.

Salensky (1895) described the embryology and the larva of a species which he called *Didemnum niveum* in the title of his paper. On the second page

(p. 289) he states, 'Nach meiner früheren Bestimmung glaubte ich, dass diese dem *D. cereum* angehörten; es waren zwar in der That einige Exemplare von dieser Species dabei, aber die Hauptmasse der Colonien, die ich studiert habe, gehören einer anderen Species an und stehen dem *D. niveum* am nächsten'. Apparently he was not altogether sure of his identification. Whatever his species was it was evidently the most abundant didemnid at Villefranche. Harant & Vernières (1933) state that *Trididemnum niveum* has only once been recorded in the Mediterranean, thus implicitly doubting Salensky's identification. Berrill (1950) includes Salensky's species under the synonyms of *T. tenerum* (= *T.* or *Didemnum cereum*), but also gives it as a reference for *Trididemnum niveum* with a note (p. 141) suggesting that Salensky may be mistaken in his identification. The larva which Salensky describes is in fact unmistakably that of *T. tenerum* and differs in many points from that of *T. niveum*. Presumably he had two different colour varieties or forms of *T. tenerum*, one of which he recognized as such ('*Didemnum cereum*') while the other he failed to ascribe to its correct species.

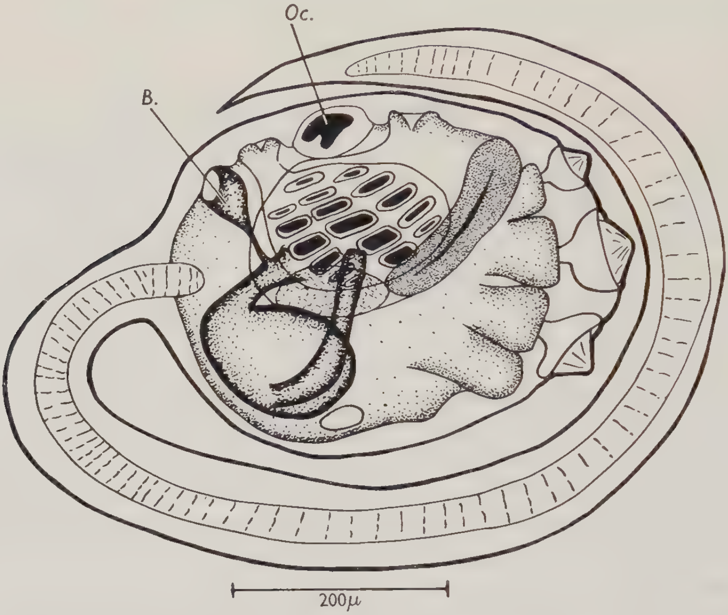

Fig. 1. Drawing from the left side of the larva of *Trididemnum niveum*. Note precocious budding and lack of otolith. *B.*, bud; *Oc.*, ocellus.

Accordingly, the larva of *Trididemnum niveum* has not been described, an omission that can now be remedied.

The length of the body of the larva of *T. niveum* is slightly greater than that of *T. tenerum*, averaging about 480 μ, while that of *T. tenerum* averages about

430 μ. Mediterranean specimens of *T. tenerum* tend to have a smaller larva than northern ones, in my experience, and Salensky's figures indicate a length of about 400 μ. The tail is comparatively longer in *T. niveum*, extending round even past the sensory vesicle as far as the atrial siphon, whereas in *T. tenerum* it rarely passes the branchial siphon, when coiled around the body just before hatching. At the time of hatching the stomach of *T. tenerum* is hardly swollen beyond the thickness of the rest of the gut; in *T. niveum* it is globular. On the other hand, the gut of *T. tenerum* already has its lumen developed by the time of hatching, whereas that of *T. niveum* is solid even up to metamorphosis. There are three suckers in both species. The ampullae number about four pairs, but departures from this number are to three pairs in *T. tenerum* and to five pairs in *T. niveum*. I have been unable to distinguish any signs of the existence of an otolith in the *T. niveum*, a feature which is shared, so far as I know, only with *Pycnoclavella* (Berrill, 1947) and has not been observed in any other didemnid. Quite a number of specimens of larvae of *T. niveum* show precocious budding from the oesophageal region. The precocity is not nearly so pronounced as in *Diplosoma*.

The free-swimming period is about 20 min.

SUMMARY

Trididemnum niveum has increased in numbers at Plymouth between spring, 1951, and March 1954, until in some localities, the species is the most abundant of ascidians. The larva is described and figured; it lacks an otolith and shows precocious budding, but otherwise is typical for the family.

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J. Mar. biol. Ass. U.K. (1954) 33, 329–334
Printed in Great Britain

STYELA MAMMICULATA N.SP., A NEW SPECIES OF ASCIDIAN FROM THE PLYMOUTH AREA

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(Text-figs. 1 and 2)

Styela mammiculata[1] n.sp. is based on four specimens collected in the Plymouth area during the summer of 1953. The most noticeable external feature is the extensive mammillation of the test. The mammillae are much smaller than those of *Phallusia mammillata* and deserve to be called rather 'mammiculae'.

Of the four specimens, two were taken from Drake's Island buoy (National Grid Reference, 20/4753) in Plymouth Sound, growing about half a metre below the water line, and two from the oyster bed of the Lynher river estuary, near Antony Ferry (National Grid Reference, 20/4257), growing about 12 m. below low-tide mark attached to oyster shells. The largest specimen was from the latter locality and measured 111 mm. long in the contracted, fixed condition. The smallest, from Drake's Island buoy, was 54 mm. long.[2]

Each specimen (see Fig. 1) is attached by an enlarged plate of test substance from which arises a short stout pedicel, accounting for about one-third of the total length of the body. It is evident that the stalked condition is not dependent on habitat, as it is in *Styela plicata* (Lesueur), which develops the stalked condition only under conditions of crowding, for of the four specimens of *S. mammiculata* two were growing (on Drake's Island buoy) in crowded conditions among *Molgula* while the other two, from the Lynher oyster beds, were quite isolated, attached to oyster shells, and yet showed the same degree of stalk development. In all four specimens the stalk is bent through a right angle so that the body comes to lie parallel to the substrate with the ventrum outwards. The animals in the Lynher must therefore have been lying horizontally, and those on the buoy probably vertically. The curvature persisted in one specimen through 3 months in the aquarium, during which time the animal doubled in size. The basal 'holdfast' is rather sharply delimited from the pedicel which is transversely wrinkled at the base, even in living animals, but longitudinally wrinkled for the greater part of its length. The body is mammillated in the upper region and transversely wrinkled, when contracted, in the lower part. The entire test is pubescent. In colour the animals are brown, with the mammillations a paler colour. The colour is retained in formalin for at least 3 months. The four-lobed atrial and buccal siphons are marked with longitudinal stripes of almost white and a rich chocolate brown, four of each. The brown stripes are rather wider than the white, and each one is divided by a central paler zone which does not quite reach to the edge of the siphon (see Fig. 1, inset). The same colour pattern is repeated on the inside of each siphon but does not continue very far in, fading to a whitish hue about 4–5 mm. down. When the siphons are open they are almost perfectly round, or at least there are no obvious angles. When they close the white stripes remain outwards, while the dark stripes bend to meet each other; the white stripes in the contracted condition show slight mammillations. An end view of a partly contracted siphon rim shows the pattern of a four-leafed clover with the sharpest angles inwards and rounded angles on the outside; this is the reverse of the more common condition in Styelidae and Pyuridae, where the partly closed siphon is frequently squarish in end view, with the most acute angles outwards.

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[1] From Latin *mammicula*, a small breast or protuberance.

[2] Since writing this paper a fifth specimen, 47 mm. long, has been collected from a buoy in the Sound and a sixth, 129 mm. long, from West Wharf, Millbay docks.

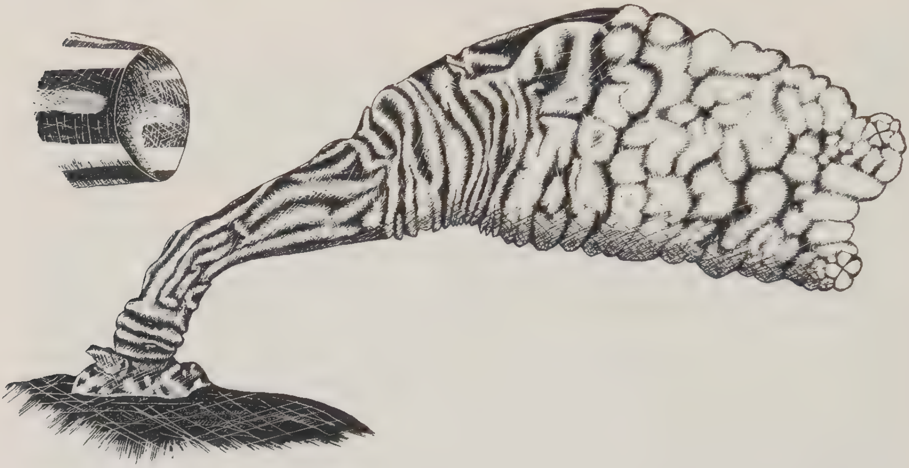

Fig. 1. Sketch of a contracted specimen of *S. mammiculata* from the left side; natural size. Inset: the buccal siphon open, showing the pattern of dark and light stripes; × 3.

The mantle separates readily from the test and is moderately stout and muscular. It extends the whole way down the pedicel, which is hollow, but not into the 'holdfast' which is solid. This character is shared with *S. montereyensis* (Dall) and *S. barnharti* Ritter & Forsyth, the stalked species of the Californian coast.

The branchial siphon has a single ring of about forty simple tentacles which are winged (Fig. 2D), but not distinctly keeled, and all of one order of size—about 4·5 mm. in the largest specimen. The dorsal tubercle is about 2 mm.

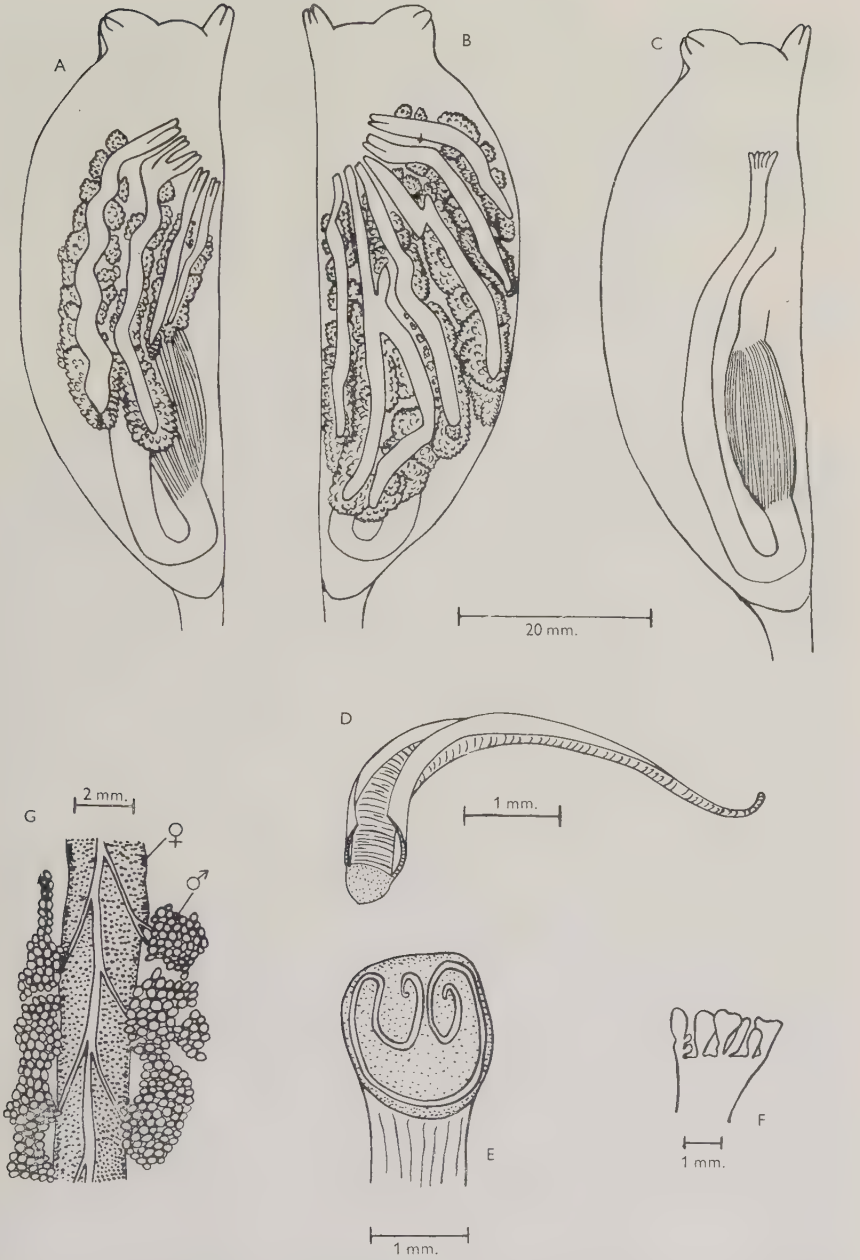

Fig. 2. Anatomical details of *S. mammiculata*. A, zooid removed from test, drawn from the left side; B, the same from the right side; C, the zooid from the left side with the mantle and gonads removed to show the course of the gut; D, an oral tentacle showing the 'wings'; E, the dorsal tubercle; F, the anus; G, a portion of a gonad.

across in the largest specimen. The opening of the ciliated pit is horseshoe-shaped, with both horns incurled in opposite directions (Fig. 2E). The open interval is directed forward and not inclined to either side. The dorsal lamina is a smooth-edged continuous membrane. It continues far behind the oesophageal opening. The branchial sac is robust and almost opaque. It bears four well-developed folds, all approximately equal in height. In the largest specimen the inner longitudinal bars show the following arrangement:

Left side: D 8 (32) 13 (36) 9 (36) 8 (30) 7 V
Right side: D 7 (30) 12 (36) 9 (32) 8 (30) 6 V

In a specimen 71 mm. long the arrangement was:

Left side: D 6 (29) 11 (30) 9 (31) 6 (29) 5 V
Right side: D 6 (29) 10 (31) 9 (30) 7 (28) 5 V

Transverse vessels are numerous and of at least two orders of size. On the flat part of the sac, between the folds, there are three to five stigmata per mesh.

The oesophageal opening is relatively far forward, in front of the middle of the body proper. The gut, which lies on the left side of the body, forms a simple U, with the oesophagus, the stomach and the proximal part of the intestine forming the descending branch, while the distal part of the intestine forms the ascending branch (Fig. 2C). The stomach is pleated with about forty longitudinal folds. The anus is frilly with sometimes secondary ramifications to the lobes (Fig. 2F). It lies behind the anterior end of the gonads.

Endocarps are numerous on the intestine and stomach as well as on the inner face of the mantle.

The gonads are of the typical *Styela* form, with each a central strand of ovarian tissue flanked by testis follicles. They number three or four (possibly five) on the left side and five, six or seven on the right. One or more may be branched. The ovarian tissue is orange in life; the colour fades over a period of months in formalin. The testis follicles are brilliant opaque white. The vasa efferentia are short so that the testicular tissue lies only just beyond the ovarian, with no clear space between. A mass of small tubular testis follicles opens into each vas efferens, arranged with their ends towards the outside of the mass and showing as a series of circles. Frequently the follicles from two or more adjacent vasa efferentia are intertangled so that it appears as if the testis ran along the side of the ovary attached here and there by vasa efferentia. The tubules are far more abundant and smaller than in any other species of *Styela* with which I am acquainted. The gonadal openings are rather far back in the atrial cavity, about a quarter of the length of the body from the atrial siphon, as an average. Those on one side are arranged along an arc of a circle.

There can be no doubt that this is a species of *Styela*. *S. mammiculata* is clearly different from any European species of *Styela* in the large number of gonads on the left side. In certain respects it approaches the Mediterranean *S. plicata*, notably in the number and macroscopic form of the gonads of the right side, but it differs in the number of gonads of the left side (2, occasionally 3, in *S. plicata*), in the arrangement of bars on the branchial folds (*S. plicata* has fewer bars and the ventral fold is smaller than the rest), in the form of the tentacles (which are usually keeled in *S. plicata*, and of more than one order of size), in the smaller number of stigmata per mesh, in the smaller, more numerous testis follicles and in the stalk. *S. mammiculata* is nearest to Van Name's (1945) description of the South Californian species of *S. barnharti* Ritter & Forsyth, but rather more different from the description of this species given by Ritter & Forsyth (1917). It differs from van Name's description of this species in the following points:

(i) The pattern of furrowing of the body is distinct. (ii) *S. mammiculata* has only about forty oral tentacles compared with 'at least seventy in large examples' of *S. barnharti*. (iii) The fourth branchial fold has only two to four less bars than the others in *S. mammiculata*, while in a specimen of *S. barnharti* of about the same size as the specimen whose bar-numbers are given above the fourth bar has thirteen to fifteen less bars than the others. (iv) The dorsal tubercle is more complex in *S. mammiculata*. (v) *S. barnharti* tends to have rather more gonads on the right side than *S. mammiculata*. (vi) The vasa efferentia of *S. mammiculata* are relatively shorter. (vii) The testis follicles are smaller and more numerous in *S. mammiculata*. (viii) In *S. barnharti* the gonads have never been reported to branch, a character found in three of my four specimens. (ix) The course of the gut is more circuitous in *S. barnharti* and the stomach is the hindmost part of the loop, whereas in *S. mammiculata* part of the intestine forms the posterior end of the gut loop. (x) The gonadal apertures and the anus are farther back in *S. mammiculata* than in *S. barnharti*.

S. mammiculata does not approach the Japanese species *S. elsa* Hartmeyer (1906) which Ritter & Forsyth regarded as nearest to *S. barnharti*.

S. mammiculata appears to be a new arrival in Plymouth waters, for an animal so conspicuous and of such large size is not likely to have been overlooked. Moreover, Mr T. R. Tozer, the Senior Scientific Assistant in charge of the specimen room at this laboratory, brought the first specimen to my notice because he had never seen anything like it in the course of his 20 years in that department. It is of course possible but rather improbable, that it may have been overlooked. It is likely that it has been imported with oysters.

The type specimen has been deposited in the British Museum (Natural History).

I wish to thank Dr R. H. Millar for his helpful criticisms of the manuscript.

SUMMARY

Styela mammiculata is a new species of ascidian from the Plymouth area. It is stalked and characterized from the remaining European species of the genus by possessing more than two gonads on the left side. Of previously described species of *Styela* it is nearest to *S. barnharti* Ritter & Forsyth from southern California.

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J. Mar. biol. Ass. U.K. (1954) 33, 335-339
Printed in Great Britain

NOTE ON THE PLYMOUTH '*NITZSCHIA*' CULTURE

By N. Ingram Hendey
Royal Naval Scientific Service

(Plate I)

For nearly fifty years a small marine organism has been cultured at the Marine Biological Association's Laboratory at Plymouth, mainly to be used as a food supply in rearing marine larval forms.

The cultures were originated by the late Dr E. J. Allen in 1907. The first record of these cultures and the methods used to maintain them were published by Allen & Nelson (1910) in a paper describing methods for obtaining persistent cultures of eighteen species of plankton diatoms. One of these cultures flourished so successfully that subcultures have been distributed to many laboratories and institutions both in Europe and America. This organism was named *Nitzschia closterium* W. Sm. forma *minutissima*. The authors, however, did not describe the organism or produce an illustration, so the combination can be considered only as a *nomen nudum*.

In 1939 the care of the cultures passed to Dr Douglas P. Wilson who published the first detailed account of the organism together with illustrations (Wilson, 1946). Wilson found the organism to be pleomorphic and described ovoid, fusiform, triradiate, and cruciform forms. After discussing these forms in great detail Wilson concluded 'Both normals [fusiform] and triradiates produce ovals by division, and the ovals so produced can multiply to form further ovals, or can grow either two or three arms, generally two, to form normal or triradiate cells' (p. 268), and 'there is no doubt that as a general rule triradiates arise from normals only through the intermediary of oval cells' (p. 251).

As Allen & Nelson (1910) did not describe the organism there is no means of knowing whether the original cultures contained the varying forms or not.

It is certain that Allen & Nelson examined the organism under the microscope, and because of its small size must have used high-power objectives to do so. It is reasonable to conclude, therefore, that the original sample contained only fusiform specimens, or that these were strongly dominant, for had the original workers noticed the triradiate ones, it is almost certain that they would have commented upon them.

Wilson (1946) states that Dr Allen had seen occasional triradiate forms in the cultures, and that Mr Clifford Dobell, who examined some old exhausted cultures during the winter of 1910–11, noticed enormous numbers of triradiate forms, and rightly concluded that they were present in the early cultures, and were not a product of long-continued artificial environment.

Recently considerable doubt has been expressed concerning the true taxonomic position of this organism and this paper describes efforts to clarify the problem. Hendey (1937) explained that *Nitzschia* Hassall (1845) is an absolute synonym of *Sigmatella* Kützing (1833), as both genera were based upon *Bacillaria sigmoidea* Nitzsch. The name *Nitzschia* was adopted until it was legally conserved, in the sense that most modern taxonomists have used it—that is, in the sense that W. Smith used it in his *Synopsis of British Diatomaceae* (1853)—taking *Nitzschia sigmoidea* W. Smith based on *Sigmatella Nitzschii* Kützing, which was *Bacillaria sigmoidea* Nitzsch, as the type of the genus.

Smith (1853) described *Nitzschia* as follows: '*Frustules* free, elongated compressed; valves linear, keeled, with one or more longitudinal lines of puncta; keel frequently eccentric.' This definition differs in no material respect from Hassall's, and, in explanatory notes that follow it, the importance of the keel, usually eccentric and punctate, is stressed as the dominant generic character.

Wilson (1946), in his description of the three phases of the organism, makes it quite clear that he was unable, with certainty, to see any of the characteristic markings associated with the genus *Nitzschia.* He states (p. 237): 'I have not been able to satisfy myself that I have seen any of the usual valve markings of the Nitzschioideae, the keel and canal raphe or the carinal dots.... Only in some gently incinerated specimens mounted in Sirax (a medium of high refractive index) were to be seen what might possibly be the keel and raphe with some slight suggestion of carinal dots, but it was impossible to be certain of the identity of the structures seen; they might very well have been artefacts.'

Commenting on the structure of the triradiate cell Wilson states (p. 237): 'It would be interesting to know how the keel and raphe, if present, are arranged, but it has not been possible to make them out.'

It is well known that some diatoms are very weakly siliceous and that definitive markings are difficult to observe, and, further, that some diatoms in culture have been induced to vacate their siliceous frustules and continue to live as naked bodies (Wiedling, 1941; Hendey, 1945, 1946), but these forms were derived from individuals possessing normal frustules. There can be no suggestion that the weakness of silicification and the consequent hyaline nature of the cell wall is attributable to culture conditions, or that a 'laboratory species' has, at some time, suddenly arisen due to conditions imperfectly known.

Wilson states (1946, p. 265), on the authority of Dr Mary Parke, also of the Plymouth Laboratory, that both the triradiate and fusiform phases were frequent in water samples from the Irish Sea off Port Erin.

It must be accepted, therefore, that there exists in nature a weakly siliceous organism having certain diatom characteristics, and that persistent cultures of it have been maintained over many years without producing any apparent change.

This polyphasic organism was ably described and illustrated by Wilson (1946) under the name of *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima* Allen & Nelson. Wilson drew attention to the fact that a unicellular alga described by Bohlin (1897) as *Phaeodactylum tricornutum* agreed in all particulars with the triradiate of the Plymouth cultures. Bohlin had found this organism in Baltic rock pools; it had occurred with *Brachiomonas submarina* Bohlin and had multiplied rapidly in culture as the *Brachiomonas* died out.

Mr Michael Droop of the Marine Station, Millport, who is familiar with Bohlin's organism which he has himself collected in the Baltic, has intimated (in a private communication) that it commonly occurs there in rock pools on the skerries, and that he has found it within a few miles of Runmarö. Further, that in his experience, naturally occurring material always has been triradiate.

Mr Droop deposited a culture of the Baltic material at the Plymouth Laboratory, and comparisons made with it and the Plymouth specimens confirm beyond all possible doubt that both should be referred to Bohlin's species.

It should be noted that Allen & Nelson (1910) did not describe or illustrate their organism, but were content to list it as an undescribed variety of a well-known species. One of two explanations seem probable. First, that Allen & Nelson made a misidentification due to lack of information on the diatoms. The fusiform organism found by them in the original Plymouth material, whatever it was, was probably unknown to them. Its general appearance suggested a relationship with *Nitzschia closterium* Ehrenberg, but they satisfied themselves that it was not the type variety, and adopted forma *minutissima*, without describing it, merely as a matter of convenience. Secondly, that the original material cultured was predominantly a small *Nitzschia closterium* with a few specimens of *Phaeodactylum* which were, most likely, not noticed because of their small size. Subsequent subculturing at short intervals favoured the *Phaeodactylum* which replaced the *Nitzschia* very much in the same way as it replaced the *Brachiomonas* in Bohlin's cultures.

Dr D. P. Wilson (in private communication) favours the former view. He states that when Dr Allen spoke of the 'normals' in the cultures, he referred to those illustrated by Wilson (1946, p. 236, fig. 1, left-hand figures), which are fusiform specimens of *Phaeodactylum tricornutum* Bohlin, and that to the best of his knowledge and belief the original cultures had contained only 'normals', i.e. fusiform organisms. Dr Wilson also assures me that on some microscope mounts made by him in 1930 the triradiate form is present in small numbers but there are many more fusiform cells with them, and that no true *Nitzschia* are there to be found. Whatever the cultures may have contained in the beginning, it is now clear that they have consisted only of *Phaeodactylum tricornutum* Bohlin in several phases for very many years.

The varying phases of the organism, ovoid, fusiform, triradiate, etc., and the ability to change one into the other, have been dealt with by Wilson & Lucas (1942) and Wilson (1946). It is not clear what factors determine the shape of the cells, but no doubt age of culture, availability of nutrients, and light intensity all play a part.

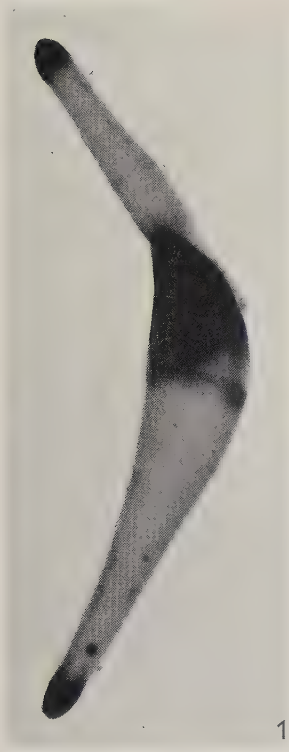
Bohlin (1897) described only triradiate specimens, Allen & Nelson (1910) indicated that only fusiform specimens were to be seen in their material. This does not exclude the possibility that some fusiform specimens were present in Bohlin's material, or that some triradiates were present in Allen & Nelson's, but only that they were not recorded. Droop intimated that his culture (on solid media) of the Plymouth organism has never given rise to triradiate forms, while a clone from the Baltic had been triradiate for most of the time since collection, but now, on solid media, contains only ovals which when subcultured into liquid media have given rise to fusiform cells but not to triradiates.

In an attempt to elucidate the problem, electron micrographs have been prepared. Pl. I, figs. 1 and 2 show the fusiform phase, and figs. 3 and 4 show the triradiate phase. The original prints of these were made at a magnification of × 10,000. The micrographs show the cell-wall to be completely hyaline and entirely devoid of any structures. The organism, as interpreted by the electron microscope, adds little to that already observed by the high-power optical microscope.

The true taxonomic position of this organism is still in some doubt. There appears to be evidence that the cells divide longitudinally as do the diatoms, but the weakly siliceous nature of the cell-wall precludes any positive observations to determine whether or not the cell is frustular. Wilson (1946, p. 237) sectioned the cells, but was unable to see any evidence that would suggest the presence of valves and girdles characteristic of diatoms.

Recently Dr Mary Parke has shown (unpublished) that leucosin is present in the cell of *Phaeodactylum tricornutum* from the Plymouth cultures. The presence of leucosin suggests that *Phaeodactylum* might be related to the Chrysophyceae, but too much importance must not be given to this suggestion without further research.

I wish to express my indebtedness to Mr F. W. Cuckow for the electron micrographs shown in Pl. I, and to Dr Mary Parke, Dr D. P. Wilson, and Mr Michael Droop for their kind assistance.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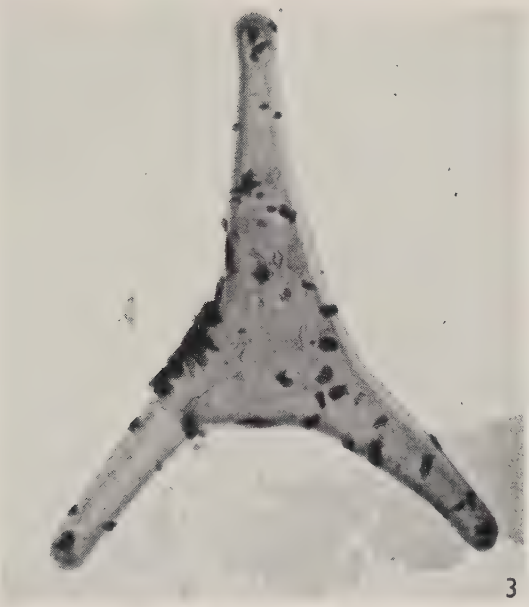
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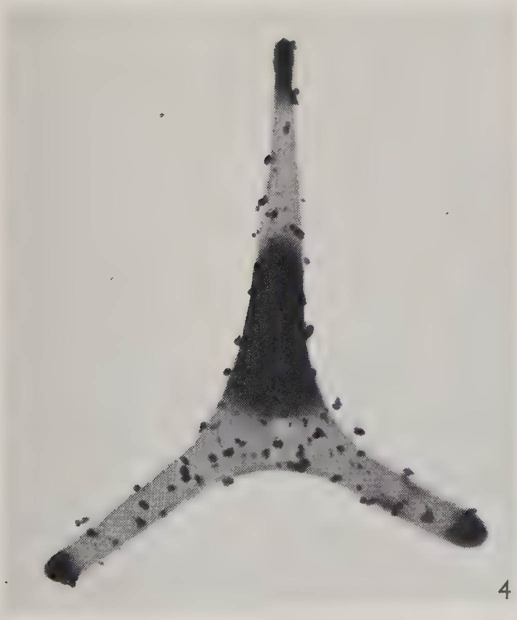
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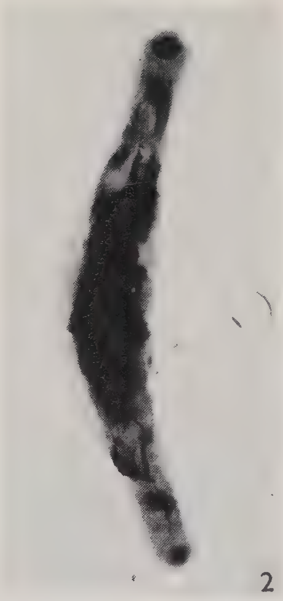
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EXPLANATION OF PLATE I

Phaeodactylum tricornutum Bohlin

Figs. 1 and 2. Cell in fusiform phase. × *c.* 6000.
Figs. 3 and 4. Cell in triradiate phase. × *c.* 6000.

J. Mar. biol. Ass. U.K. (1954) 33, 341–344
Printed in Great Britain

PRELIMINARY NOTE ON A SURVEY OF STOKE POINT ROCKS WITH SELF-CONTAINED DIVING APPARATUS

By G. R. Forster

The Plymouth Laboratory

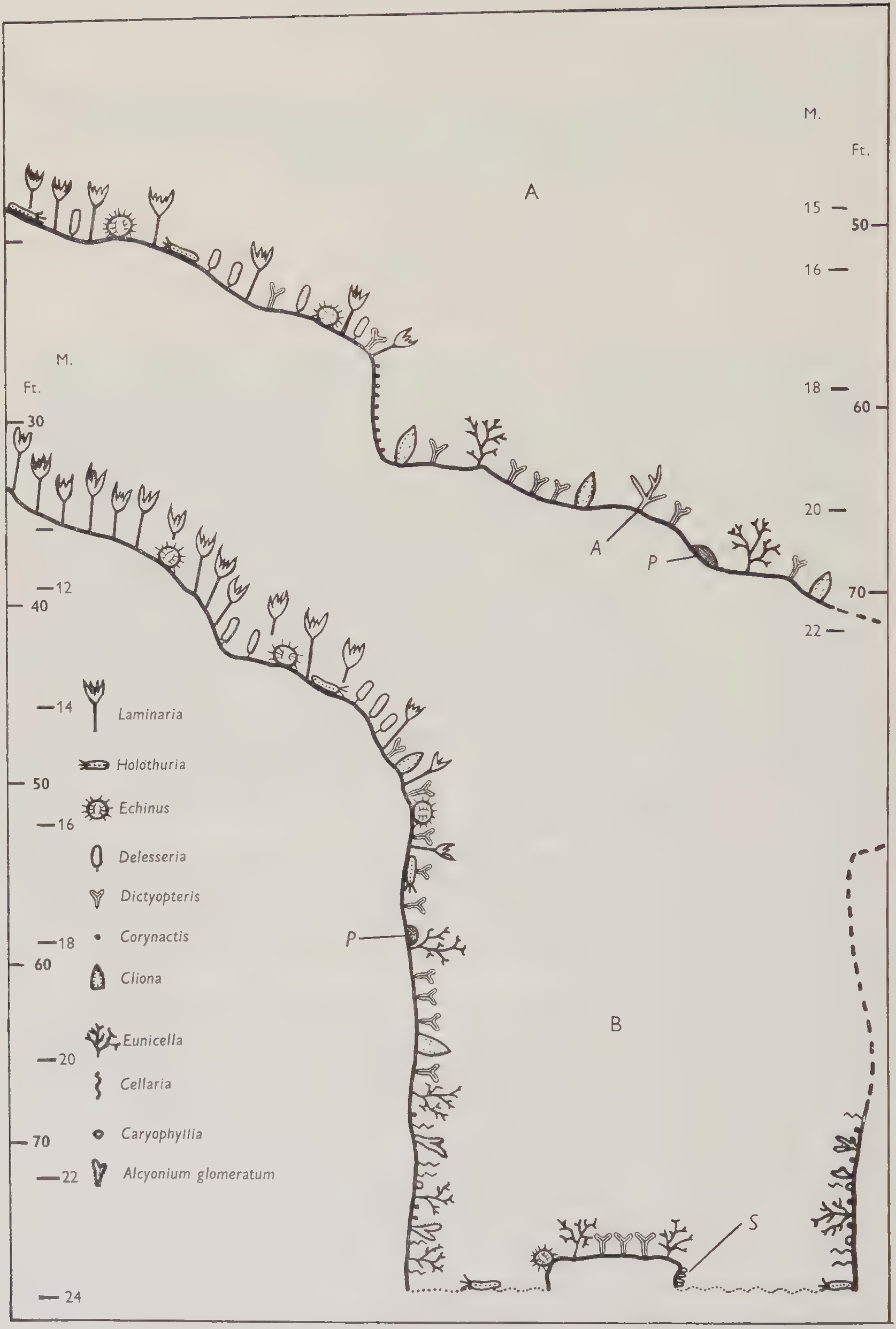
(Text-fig. 1)

It has long been recognized that a proper knowledge of the fauna of submerged rocks can be obtained satisfactorily only by direct study with diving apparatus (Gislen, 1930; Kitching *et al.*, 1934). Although the latest methods of indirect underwater observation, photography and television, may be useful on a smooth sea floor, they can give little information about a rocky area owing to their inability, as yet, to look underneath a boulder or an overhanging ledge. The superiority of the 'submarine biologist' over the indirect methods lies in the fact that he can readily collect samples for positive identification, or mark individual organisms for further study; though at present, unless very expert, he is limited to depths of under 20 fathoms. The successful use of free-diving apparatus for biological work is well established. Five years ago Prof. Drach with Cousteau's now famous 'aqualung' investigated the sublittoral fauna of various rocky shores, reaching a depth of 30 m. (Drach, 1948). Much additional work has been done in the Mediterranean and other warm seas, while farther north Bainbridge (1952) used a Siebe-Gorman apparatus to study the behaviour of plankton in the Clyde.

My training was carried out at the Portsmouth and Plymouth Royal Naval diving establishments. In 1952 a Siebe-Gorman 'aqualung' was purchased with the aid of a Royal Society apparatus grant. This breathing set, combined with a S.-G. light two-piece waterproof suit formed the equipment used during a survey in 1953.

The area of Stoke Point rocks was selected as being the most suitable locality for studying the fauna and flora which replace *Laminaria hyperborea* (Gunn) Fosl. at a depth of about 10 fathoms (see Chapman, 1944), since in less than 100 yards the depth increases from 6 to 12 fathoms. The exact position is: distance from Stoke Point, MHWST, 0·40 sea mile, bearing 146° T. After considerable practice, the sub-*Laminaria* zone was reached in August 1953, but only six dives were made during the period from August till October, owing to bad weather restricting the already limited opportunities to use the research vessels. The results are thus based on a total period of but 2 hours 'diving time' and refer only to the larger and more conspicuous organisms.

The results are shown diagrammatically in Fig. 1. *Laminaria hyperborea* is


M.
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A
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50
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22
M.
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30
12
40
14
Laminaria
Holothuria
50
Echinus
16
Delesseria
Dictyopteris
18
Corynactis
60
Cliona
Eunicella
20
Cellaria
70
Caryophyllia
22
Alcyonium glomeratum
24
P
B
S

Fig. 1. Diagrammatic profiles of rocks at Stoke Point, S. Devon. The depths are given as feet and metres below chart datum, i.e. MLWST Devonport. The horizontal scale is approximately the same.

A, profile of reef 100 yards north-west of gully;

B, profile of the gully. Key inset above. *A*, *Axinella*; *P*, *Pachymatisma*; *S*, *Stolonica socialis*.

clearly the dominant form to a depth of 50 ft. below low water (C.D.). At 35–40 ft. a few *Echinus esculentus* L. appear, together with *Holothuria forskali* Delle Chiaje, though unlike *Echinus*, *Holothuria* is occasionally taken intertidally in the Plymouth area. Below 50 ft. the *Laminaria* is sparse and *Delesseria sanguinea* (Huds.) Lamour. becomes abundant, both plants being heavily encrusted with Bryozoa. The *Delesseria* is replaced at about 55 ft. by a thin carpet of the brown weeds, *Halopteris filicina* (Grat.) Kütz. and especially *Dictyopteris membranacea* (Stackh.) Batt., neither of which suffer from Bryozoa or epiphytes. At this depth large yellow colonies of the sponge *Cliona celata* Grant are seen, together with the smooth grey *Pachymatisma johnstoni* Bowerbank, which in shallower water is confined to crevices or shaded positions. The conspicuous pink or flesh-coloured colonies of *Eunicella verrucosa* (Pallas) appear at around 65 ft. These 'sea fans' were always orientated in a nearly vertical plane, thereby presenting the maximum area to the tidal stream. In the gulley (Fig. 1 B) *Eunicella* reaches a maximum frequency of very roughly 2–4 per square metre, from 10 ft. above to 1 ft. above the bottom: the lowermost foot was, at least in several places, completely bare due presumably to abrasion by the coarse sand of the gulley floor. In this area where the *Eunicella* flourishes, the *Dictyopteris* is distinctly sparse, the ground layer being composed largely of Bryozoa, especially *Cellaria* sp., some encrusting sponges and many *Corynactis viridis* Allman. Almost as numerous as the *Eunicella* is *Alcyonium glomeratum* (Hassal), whose snow-white polyps make a bright contrast against the otherwise deep red surface of the colony. The rarity of this species in dredgings is probably explained by the fact that it has not been seen outside the gully. At the same depth as the *Alcyonium* and *Eunicella*, a few large solitary ascidians *Phallusia mamillata* (Cuvier) appear, and occasional colonies of ross (*Lepralia pallasiana* (Moll.)), the tubicolous polychaete *Filograna implexa* (Berkeley) and the yellow branching sponge *Axinella dissimilis* (Bowerbank).

SUMMARY

A preliminary survey has been made, near Stoke Point, S. Devon, of the rock fauna at depths below the *Laminaria* belt, with self-contained compressed-air diving apparatus. By this method much more exact records of the distribution, and general abundance of the commonest species, have been obtained than would be possible with dredgings. It is hoped to make a greatly extended survey in the future.

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J. Mar. biol. Ass. U.K. (1954) 33, 345–346
Printed in Great Britain

WARM-WATER SPECIES IN THE PLANKTON OFF THE ENGLISH CHANNEL ENTRANCE

By J. H. Fraser, D.Sc.
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Through the kindness of Dr L. H. N. Cooper of Plymouth I have been given the opportunity of examining some of the plankton samples taken by Surg.-Lt. P. Campbell, R.N.V.R., of H.M.S. *Challenger*. It is hoped that the results of these and later surveys will be published elsewhere in more detail, and in association with those made by the Scottish research vessels farther north, but two records of unusual interest are brought to notice here. The collections so far examined in detail were taken during April and May 1953, on a line of stations west of the English Channel, from 49° 39′ N., 3° 30′ W. to 47° 16′ N., 17° 52′ W. between 15 and 19 April, and on the return line from 47° 50′ N., 17° 40′ W. to 49° 28′ N., 5° 52′ W. between 25 and 28 May. They were made by 10 min. horizontal hauls at 30 fathoms depth.

On 17 April 1953, at 47° 31′ N., 14° 21′ W. the plankton contained a rich variety of oceanic species consisting mainly of various Siphonophora. Amongst them were found a single specimen of the solitary form of the salp *Ritteriella picteti* Apstein, 15 mm. long, and seven specimens of the aggregate form of *R. amboinensis* Apstein, 10–25 mm. long. No other salps were found there.

This specimen of the solitary form of *R. picteti* has sixteen body muscles, all interrupted on the ventral side, the range for this species being from thirteen to twenty-one or more. It is clearly distinguishable from the solitary form of the closely allied *R. amboinensis*, which usually has only eleven muscles of which the first three are continuous ventrally, but may have from ten to thirteen. There are also other differences, particularly in the form of the gut. The specimen is in excellent condition and was obviously thriving when caught; it is a young one as this species can reach at least 70 mm. in length.

The seven aggregate specimens of *Ritteriella* found by H.M.S. *Challenger* have muscle arrangements exactly corresponding to the published description of *R. amboinensis*, including the arrangement of muscle VI which, according to Thompson (1948), is not known in other *Ritteriella* species or in the *Cyclosalpa* group. The aggregate form of *Ritteriella picteti* is imperfectly known. One very small embryo taken from the stolon of a solitary form and figured by Apstein as *R. amboinensis* was later ascribed by Ihle to *R. retracta*, now thought to be synonymous with *R. picteti*. Three small (3–4 mm.) specimens from Australian waters were ascribed with some doubt to *R. picteti* by Thompson (1948), who also took embryos from the stolon of a solitary form. It would appear from Thompson's description to have five dorsally approaching muscles, as in *Salpa cylindrica*, and would thus be sufficiently distinct from that of *Ritteriella amboinensis* to make recognition possible. These *Challenger* specimens are in quite good condition, and their identification as *R. amboinensis* and not *R. picteti* would, according to this, seem reliable, although more work and material is required before the relationship between these species can be adequately understood.

Both these species are considered to be equatorial and are regarded as rather rare even there, especially *R. picteti*. Both occur in the Pacific Ocean, particularly in the neighbourhood of the East Indies and Philippines, and in the Indian Ocean. *R. amboinensis* has been recorded in the equatorial and southern Atlantic, off the coast of Africa, but because of a confusion in synonymy it is doubtful if *R. picteti* has yet been recorded from the tropical Atlantic. Neither species has previously been recorded from the north-temperate Atlantic.

The same area was re-examined by H.M.S. *Challenger* towards the end of May, and in the interval the total plankton had decreased and only a few siphonophores were taken, with some *Salpa fusiformis*. However, at 47° 40′ N., 13° 58′ W. on 26 May, i.e. only a few miles from the earlier station referred to, a specimen of a very large *Coscinodiscus* type of diatom, 1·8 mm. in diameter, was taken. This was identified for me by Mr R. Ross of the British Museum (Natural History) as *Ethmodiscus gazellae* (Janisch) Hustedt, which is the largest diatom known and is recorded up to 1·9 mm. in diameter. He tells me that this species is widespread in tropical waters, being specially common in the Pacific, and that it has been recorded from the Mediterranean (Hustedt, 1930, pp. 374–6) and from the Cape Verde Islands (Castracane, 1886, as *Ethmodiscus gigas*). This is believed to be the first record of its presence in the north-east Atlantic. The specimen was in excellent condition and obviously alive when caught.

The abundance of 'Mediterranean' or 'Lusitanian' species in the plankton west of the British Isles has been remarkable in 1953, and will form the basis of a more detailed report elsewhere.

The specimens of *Ritteriella picteti* and *R. amboinensis* are being held at the Marine Laboratory, Aberdeen; the *Ethmodiscus gazellae* is deposited with the British Museum.

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J. Mar. biol. Ass. U.K. (1954) 33, 347–360
Printed in Great Britain

BIOLOGICAL DIFFERENCES BETWEEN SEA WATERS: EXPERIMENTS IN 1953

By Douglas P. Wilson, D.Sc. and F. A. J. Armstrong
The Plymouth Laboratory

(Text-fig. 1)

The experiments during the 1953 season were arranged to investigate certain criticisms and suggestions made to us after publication of the earlier work (Wilson, 1951; Wilson & Armstrong, 1952), and there were included tests of our own devising intended to narrow the field within which must be made any search for an explanation of the observed biological differences between sea waters from different localities. We were again unable to obtain water from the Celtic Sea and once more, by kind co-operation of the Millport Marine Station, used water from the Firth of Clyde instead. Both the E 1 and Clyde waters were collected on the same day and the fertilizations of *Echinus esculentus* were made and the experiments started 4 days later, which was the minimum time possible after collection of the Clyde water. Every experiment included a control comparison of the two waters.

One of the more interesting investigations last year was the effect on the larvae of *Echinus* of water passed through active carbon. Unfortunately further investigation, as detailed below (p. 351), showed that the sample of carbon used contained a significant amount of copper, and the results obtained may be therefore attributable to the presence of that metal. This was found by analysis of the carbon and checked by analysis of sea water passed through it and, later, by the addition of copper to sea water in a section of Exp. I below. Messrs Sutcliffe, Speakman and Co., on being informed of our observations, very kindly supplied a sample of active carbon substantially free from copper with which we were able to repeat last year's experiment with different results.

In a review (*J. Conseil*, Vol. 18, p. 249) of a paper by Loosanoff and others and also in private correspondence, Dr H. A. Cole pointed out that no attention has been paid to the possibility that differences between waters may be due to bacterial activity during the period between collection and use. He suggested that, to test this, water should be filtered free from bacteria at the time of collection. This we have endeavoured to do as recorded below.

That extracts from adult *Echinus* could supply substances needed by the developing larva was a possibility worth testing. Water in which larvae had decayed and presumably released materials into the water was filtered and tested.

From time to time it has been suggested to us by various people that vitamin B_{12} would be likely to benefit larvae developing in water to which it had been added. We are grateful to Dr A. P. Orr for informing us that at Millport improvement in fertilization of *Echinus* had been effected by the action of E.D.T.A. in sequestering heavy metals and for referring us to work by Tyler (1953). Tyler claims a significant increase in the life-span of sperm pre-treated with versene (E.D.T.A.)—which he used amongst other sequestering agents—as well as an improvement of the fertilization reaction.

The stability to heat of the factor responsible for the differences between waters was tested by heating the waters at boiling-point and also by autoclaving.

Half a century ago Herbst (see list of papers in Needham, 1931) in a series of papers described the different effects obtained by rearing echinoderm larvae in artificial sea waters from which he omitted one normal constituent. We decided to observe the effect of rearing the larvae in an artificial sea water known to contain all normal inorganic constituents, because the factor we are seeking is most probably not one of these.

Methods

Cleaning of Apparatus

The methods used in the earlier experiments were followed. Glassware was cleaned with hot 1:1 sulphuric acid, and the filtering apparatus was sterilized in an autoclave and washed with boiled distilled water before use.

Collection of Water

Water from the International Hydrographic Station E 1 was taken from the sea surface with a wooden bucket and was strained through 200-mesh bolting-silk into a cleaned carboy. Clyde water was taken in the Largs channel near Millport, being dipped from the sea in a glass breffit and strained through 200-mesh bolting-silk into a carboy cleaned and dispatched from Plymouth.

Collection of Bacteria-Free Water

The most practicable method seemed to us to be one in which water was drawn through a bacteriological filter into a sterile bottle. The apparatus had to be portable and simple. The first arrangement tried was that shown in Fig. 1 A. After cleaning, the Winchester bottle was evacuated at a water pump, the rubber tube being disconnected. The tap was then closed, the tube and filter candle attached, and the whole arrangement was sterilized by heating in an autoclave at 10 p.s.i. pressure for 30 min. The stopcock was lubricated with a silicone grease which did not run when heated. To collect water the filter candle was immersed in a bucket of sea water and the tap opened to allow the water to be drawn through the candle into the bottle. On test, the apparatus held a vacuum for a week in the laboratory and the sterilization was satisfactory. In use, however, the stopcocks leaked, possibly because of vibration in transit, and the arrangement of a sealed breakable glass tube shown in Fig. 1 B was substituted. Although this avoided leakage, the samples were still not sterile. We think it likely that the Berkefeld filter candles were not of a sufficiently fine porosity. On one occasion, however, a sample was obtained which was sterile by our tests, and the experiments show that no obvious harmful effects are caused by the rubber or ceramic of the filter apparatus.

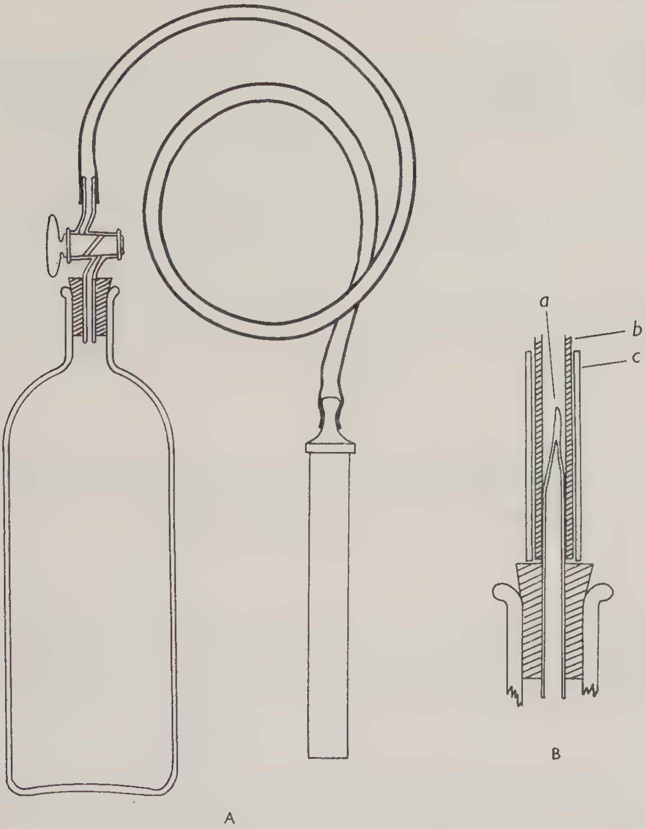

Fig. 1. A, Winchester bottle with filter candle attached for collecting filtered water. B, sealed tube arrangement to replace stopcock: (*a*) glass tube sealed after evacuation of bottle; (*b*) rubber tubing; (*c*) loosely fitting thick glass sleeve to protect tip of A. The apparatus is evacuated, sealed and assembled, and then sterilized. In use the sleeve *c* is slid up the rubber tube and the tip of *a* broken off inside *b*.

Sterility Testing

We are indebted to Dr C. H. Jellard, Public Health Laboratory Service, for his help and interest in this part of the work. Dr Jellard himself carried out most of the inoculations. On one or two occasions one of us (F. A. J. A.) did them.

The media used were: (i) peptone sea water, (ii) peptone sea-water agar, (iii) casein sea-water agar, as described by Spencer (1952).

Tubes and Petri dishes were incubated at 22° C. The sample considered satisfactorily sterile showed no bacterial growth after 3 weeks.

Heat Treatment of Sea Water

Experiment 1. One litre of each sample was heated just to boiling for 1 hr. The samples were cooled and aerated thoroughly and the volume adjusted. The treatment raised the pH from 8·04 to 8·45 for E 1 water and from 7·99 to 8·48 for Clyde water.

Experiment 4. One litre of each sample was heated rapidly just to boiling, and then cooled as quickly as possible. To avoid the high pH noted in the first experiment, acid was added. After addition of 3 ml. of 0·1 N-HCl and aeration, the pH values were E 1 8·20, Clyde 8·25.

Experiment 5. One litre of each sample was heated in an autoclave at 25 p.s.i. pressure (130° C.) for $1\frac{1}{2}$ hr. After addition of 2 ml. 0·1 N-HCl and aeration the pH values were E 1 8·01, Clyde 8·12.

Addition of Vitamin B_{12} *(Cobalamine)*

Vitamin B_{12} from Messrs British Drug Houses Ltd. was used in a concentration of 1·1 μg./l., 1 ml. of an aqueous solution containing 1·1 mg./l. being added to 1 l. of sea water.

Addition of Copper

The concentration used was 100 μg. Cu/l., 1 ml. of a standard solution of cupric sulphate containing 100 mg. Cu/l. being added to 1 l. of sea water.

Addition of Iron

The concentration used was 100 μg. Fe/l., 1 ml. of a standard solution of ferric citrate containing 10 mg. Fe/l. being added to 100 ml. of artificial sea water.

Physiological Fluids

A mixture of equal parts of coelomic fluids from male and female animals was used. Ovaries and testes were separately squeezed between glass plates, and the expressed liquids diluted with equal volumes of water. These fluids were filtered just before use, first on Whatman No. 41 (loose texture) filter-papers, and then on 'Gradocol' membranes, A.P.D. 0·49 μ.

Water in which Larvae had Died and Decayed

This was from the beaker of E 1 water in Exp. 1. After filtration on No. 41 paper and on a membrane it had a strong fishy but not unpleasant smell. It was well aerated before use.

Carbon Extracts

After the 1952 experiments an attempt was made to identify the material obtained by acetone extraction of active carbon through which sea water had been passed. Copper as cupric chloride was found, in an amount too large to have come from the sea water. On examining the carbon, it was found to contain some 1500 p.p.m. of copper, in a form which was insoluble in acetone, but which became partly soluble after the carbon had been wetted with sea water. Moreover, on passing sea water through a column of the carbon, the copper content of the water increased from 6 μg./l. (a normal value) to 60 μg./l. We consulted Messrs Sutcliffe, Speakman and Co., who very kindly supplied a sample of their carbon no. 315. On analysis it showed only a trace of copper, and no increase of the copper content was found when sea water was passed through this carbon.

This carbon was used to repeat the 1952 extraction experiments. Extracts of similar appearance were obtained. Copper was not detected in them.

Artificial Sea Water

The salts listed in the formula of Lyman & Fleming (1940) for the hypothetical combination of ions in sea water were used. After aeration the pH was 8·02.

Ethylene Diamine Tetra-acetic Acid

The disodium dihydrate (Versene, Trilon B, Sequestrine, SETate, etc.) referred to here as E.D.T.A. was used as an approximately 0·1 M solution (37·2 g./l.). Addition of 10 ml. of this solution to 1 l. of sea water gave a concentration of approximately 0·001 M or 370 mg./l., and decreased the pH to between 5 and 6. After addition of 18 ml. 0·1 N-NaOH and thorough aeration the pH values were, in Exp. 4, E 1 8·12, Clyde 8·17.

In Exp. 5, the procedure was altered slightly to minimize salinity changes. The 0·1 M E.D.T.A. solution was made up in artificial sea water, which was then adjusted to pH 8·66 by adding sodium hydroxide. 10 ml. of this solution were added to 1 l. samples of the various sea waters, giving the same concentration (0·001 M) of E.D.T.A. as in Exp. 4. Only 3 ml. of 0·1 N-NaOH were then needed to adjust the pH to suitable values. These were E 1 8·07, Clyde 8·06, artificial sea water 8·14.

Antibiotics

Penicillin G (Benzyl Penicillin B.P.C.) Glaxo, and Streptomycin (calcium chloride) Glaxo were used, one mega-unit of each being dissolved in 5 ml. of distilled water. Small volumes of this solution were measured with a microburette into 100 ml. portions of Clyde water to give concentrations of 100, 300 and 500 units/ml.

Fertilizations

These were always made in a mixture of equal parts of Berkefeld filtered Clyde and E1 waters, one male and one female being used, both carefully selected from a number of recently trawled sea urchins (*Echinus esculentus* L.).

After fertilization the eggs were immediately divided equally between two beakers, and each portion washed six times with one of the two waters. Eggs were distributed equally among the experimental dishes by the dipper method already described (Wilson, 1951, p. 5).

EXPERIMENTAL RESULTS

In every experiment at least four and often five dishes of each kind of water were tested. It should be emphasized that, unless noted to the contrary, larvae in all the dishes comprising a set of one kind of water were identical one with another. This uniformity within a set was very striking, and it was most unusual for any variation to appear before the fifth or sixth day. When differences between individual dishes within a set began to be noticeable the experiment was ended. This rarely happened before the larvae were obviously dying off.

Experiment 1

E1 water collected 16. iii. 53. Ship: R.V. *Sabella* with F.A.J.A. Salinity, 35·34; pH 8·04; temperature at time of collection, 8·5° C.

Clyde water collected 16. iii. 53. Small boat with Mr E. Latham. Salinity, 33·30; pH 7·99; temperature at time of collection, 7·1° C.

The sea-urchins were trawled on 19 March 1953 and kept under circulation until the fertilization was made on the following day. Out of about twenty sea-urchins opened none had completed spawning and only two were partially spent. A good fertilization was obtained from one ripe male and one ripe female, although a small percentage of the ova did not fertilize.

The following waters were tested. Each set comprised five dishes.

Set	
I	Clyde water
II	E1 water
III	Clyde water Berkefeld filtered at sea
IV	E1 water Berkefeld filtered at sea (bacteria-free until used)
V	Clyde water heated
VI	E1 water heated
VII	Clyde water + vitamin B_{12}
VIII	E1 water + vitamin B_{12}
IX	Clyde water + Cu
X	E1 water + Cu

The larvae did better in the Clyde water (Set I) than in the E1 water (Set II), but the difference was small compared with the more striking differences obtained in previous years. Nevertheless, it was clear that in the E1 water there were more abnormal and stunted larvae than in the Clyde water and that the best plutei in the latter were more finely developed than the best in the E 1 water. When 2 days old the larvae in the Clyde water swam a little more strongly than those in the E 1 water, but there was never at any time a marked concentration up at the surface such as had often occurred in the better waters of previous experiments. On the sixth day (5-day-old larvae), when the larvae in all sets were dying, those in the E 1 water survived for a few hours in slightly better condition than those in the Clyde water.

It is worth mentioning that the two beakers, into which the eggs were divided after fertilization, had been kept as a check on the dishes. The concentration of larvae in these beakers was very much greater than in the dishes, and the difference between the two waters was here more marked. In the Clyde water the larvae swam more strongly and survived longer than those in the E 1 water.

Of the waters filtered through Berkefeld candles at sea (Sets III and IV) only that from E 1 proved to be bacteria-free when tested just before use. The Clyde water similarly filtered, and the waters in the carboys (which after filtration supplied Sets I and II) gave heavy bacterial growths on the test plates. The larvae in the E 1 water which had been bacteria-free between collection and use (Set IV) were no different from those in the other E 1 water (Set II) and were inferior to those in the Clyde waters. The larvae in the Clyde water filtered at sea (Set III) were no different from those in the control Set I and were, of course, better than those in both the E 1 waters (Sets II and IV). Incidentally the water in Set III had a thin oily film, derived presumably from the rubber connexions of the apparatus.

The Clyde water which was heated (Set V) was barely inferior as a rearing medium to that which had not been so heated, but the E 1 water after heating (Set VI) produced decidedly poorer larvae than did the unheated E 1 water.

The addition of vitamin B_{12} to the waters (Sets VII and VIII) made no difference whatever to the appearance of the larvae.

The water with copper proved to be poisonous; in the E 1 water (Set IX) most deaths took place during early cleavage, but in the Clyde water (Set X) mainly during the blastula stage.

Experiment 2

Particulars of E 1 and Clyde water as for Exp. 1.

Fertilization made 27 March 1953 from sea-urchins trawled the previous day. Of the twenty opened only one or two were partially spent. About 100% of the ova fertilized, but nearly half cleaved irregularly and gave misshapen blastulae.

The following waters were tested. Each set comprised three dishes.

Set	
I	Clyde water
II	E1 water
III	E1 water + filtered coelomic fluid from an adult *Echinus*
IV	E1 water + filtered ovary juice from an adult *Echinus*
V	E1 water + filtered testis juice from an adult *Echinus*
VI	75 ml. E1 water + 25 ml. filtered E1 water from Set IV of Exp. 1 after death and decay of the larvae

In the Clyde water (Set I) the larvae did a little better at first than in the E1 water, but by the fifth day they were a little better in the E1 water, repeating more or less the result in Exp. 1. Again the beakers showed the most marked difference, the Clyde water giving the most vigorous larvae and the longest survival.

In all instances the additions (Sets III–VI) had slightly unfavourable effects on the development of the larvae, and each kind of addition had an effect a little different from any of the others. It is possible that the experiment was to some extent adversely affected by the large proportion of eggs in all dishes which failed to develop properly and died and decayed early.

Experiment 3

E1 water collected 13. iv. 53. Ship: R.V. *Sabella* with F.A.J.A. Salinity, 35·30; temperature at time of collection, 8·7° C.

Clyde water collected 13. iv. 53. Small boat with Mr N. Thomson. Salinity, 33·30.

In addition to the usual carboys of water strained through bolting-silk one Winchester of water filtered through a Berkefeld candle (see p. 348) was obtained from each locality. Unfortunately the glass stopper of the Clyde Winchester was broken on the journey back to Plymouth and half the water leaked away through a packing of sterilized cotton-wool. The water remaining in the Winchester from the Clyde proved to be heavily contaminated with bacteria, as did indeed also that from E1. These waters therefore can only be regarded as having been more efficiently freed from the larger plankton organisms at the time of collection than were the waters in the carboys.

The sea-urchins were trawled on 16 April and kept under circulation until a fertilization was made the following day. The following waters were tested. Each set comprised five dishes.

Set	
I	Clyde water
II	E1 water
III	Clyde water filtered when collected
IV	E1 water filtered when collected
V	Clyde water + Clyde extracts
VI	Clyde water + E1 extracts
VII	Clyde water + blank extract
VIII	Clyde water through carbon
IX	E1 water + Clyde extract
X	E1 water + E1 extract
XI	E1 water + blank extract
XII	E1 water through carbon

Apart from Sets III and IV the experiment was a repetition of Exp. III of 1952 (Wilson & Armstrong, 1952, pp. 344–7) in which an attempt had been made to extract a growth factor. As already explained (p. 351) the active carbon used for this purpose was later discovered to contain a significant proportion of copper, which, while not appearing in the control blank extracts, affected the sea water passed through it and rendered certain conclusions invalid. In the present experiment an active carbon free from copper was used.

Throughout this experiment very little difference was discernible between the control Clyde and E 1 waters, Sets I and II, but the Clyde water had slightly better plutei until the fourth day, after which the plutei in the E 1 water were in a very slightly better condition. Some larvae in all dishes were dead on the fifth day and by the sixth day conditions generally were bad and the experiment was ended. At no time in either water were the plutei as well formed or as vigorously swimming as those in most previous experiments. It is not possible to be sure whether this was due to the condition of the waters or to unripeness of the eggs or sperm. About 100% of the eggs fertilized but a small proportion cleaved irregularly.

In the water collected through Berkefeld filters that from the Clyde (Set III) had a slightly milky appearance, but nevertheless produced larvae almost as good as those in the Clyde water control (Set I). The larvae in the E 1 water filtered when collected (Set IV) were indistinguishable from those in the E 1 control (Set II).

As in the similar experiment the previous year the carbon extracts (Sets V, VI, IX and X) proved poisonous, while the blank extracts (Sets VII and XI) made very little difference to the waters to which they were added, the larvae in these dishes being very little inferior to those in the controls (Sets I and II). The important deviation from the results of 1952 is that the larvae in the waters which had passed the active carbon (Sets VIII and XII) were indistinguishable from those in the controls (Sets I and II), whereas in 1952 they were mainly abnormally formed and most of them died early. The 1952 result must now be attributed to the presence of a toxic amount of copper, derived from the carbon, in the waters which had been passed through it.

Experiment 4

E 1 water collected 27. iv. 53. Ship: M.F.V. *Sula* with F.A.J.A. Salinity, 35·17; pH 8·06; temperature at time of collection, 9·2° C.

Clyde water collected 27. iv. 53. Small boat with Mr E. Latham. Salinity, 34·01; pH 8·35.

Two Winchesters of water filtered through Berkefeld candles were taken at each locality.

The sea-urchins were trawled on 1 May and the fertilization was made on the same day. On this date less than a quarter of the urchins opened had shed their genital products. In comparison with previous years this indicated a late spawning season.

The following waters were tested. Each set comprised four dishes.

Set	
I	Clyde water
II	Clyde water, filtered when collected (Winchester A)
III	Clyde water filtered when collected (Winchester B)
IV	Clyde water heated for a short period
V	E1 water
VI	E1 water filtered when collected (Winchester C)
VII	E1 water filtered when collected (Winchester D)
VIII	E1 water heated for a short period
IX	Clyde water + E.D.T.A.
X	E1 water + E.D.T.A.
XI	Aquarium water
XII	Artificial sea water

The experiment was originally planned to contain sets of Clyde and E 1 waters after heating in an autoclave for comparison with the same waters heated for a short period. Unfortunately the samples were accidentally spoilt during preparation and could not be used.

Throughout the 4 days' duration of the experiment (the larvae were everywhere dying when 5 days old) there was little to choose between larvae in the Clyde and E 1 controls (Sets I and V). Blastulae in the Clyde water swam up a little in advance of those in the E 1 water. At an age of 3 days there were more slightly abnormal larvae in the E 1 water than in the Clyde. The difference was just sufficiently marked for an independent observer to distinguish between them after a careful comparison. Thus the two waters, so far as their effects on the larvae were concerned, were almost identical. Although almost 100% of the eggs developed with very few abnormalities the larvae never showed any concentration near the surface as is usual with good cultures in good water. The room temperature rose slowly from 15·2 to 19·0° C. during the experiment; for the first 2 or 3 days when surface concentrations normally take place it was not unduly high. In view of past experience the temperature is not regarded as having induced weakness in the larvae, in fact they swam most strongly when 3 days old at a temperature of 18° C. The lack of vigour must therefore be due either to eggs or to sperm in poor condition, or to the condition of the waters. As the fertilization appeared initially to be good (well-raised fertilization membranes, even cleavage of almost all the eggs) it is reasonable to suspect that neither water was a perfect medium for the development of *Echinus*.

All the waters filtered at the time of collection (Sets II, III, VI and VII) gave heavy bacterial counts and the larvae reared in them differed in no way from those in the controls (Sets I and V), except Set VI where all larvae were always in poor condition with many dying at an early age. The reason for this has not been discovered, but it is thought that this water must in some way have become contaminated. The Berkefeld filter candle used was observed to have a dark stain, which while defying analysis may have been due to some toxic substance. Heating the waters for a short period (Sets IV and VIII) made no difference to the Clyde water but it very slightly worsened the E1 water.

The addition of E.D.T.A. (Sets IX and X) did not improve either water; in fact the plutei showed slight abnormalities compared with those in the controls (Sets I and V).

The plutei in the aquarium water (Set XI) were always structurally inferior, with shorter arms, to those in the controls (Sets I and V) but they swam higher in the water and on the last day had fewer arm rods protruding from the shrinking flesh.

The plutei in the artificial sea water (Set XII) were decidedly small and stunted, but in the final stages did not degenerate as quickly as those in the Clyde and E1 waters.

Experiment 5

E1 water collected 11. v. 53. Ship: R.V. *Sabella*, with F.A.J.A. Salinity, 35·16; pH 8·08; temperature at time of collection, 10·50° C.

Clyde water collected 11. v. 53. Small boat with Mr E. Latham. Salinity, 33·28; pH 8·07.

The sea-urchins were trawled on 12 May, and kept under circulation until a fertilization was made on the following day. It was found that the majority had spawned or were partially spent. A good fertilization with almost 100% normal cleavage was obtained.

The following waters were tested. Each comprised a set of four dishes.

Set	
I	Clyde water
II	E1 water
III	Clyde water + E.D.T.A.
IV	E1 water + E.D.T.A.
V	Artificial sea water
VI	Artificial sea water + Clyde water
VII	Artificial sea water + E1 water
VIII	Artificial sea water + E.D.T.A.
IX	Artificial sea water + Fe
X	Clyde water autoclaved
XI	E1 water autoclaved

The eggs for Sets V–IX were washed in artificial sea water before being placed in the dishes.

In addition to the above some tests with penicillin and streptomycin were made, each in strengths of 100, 300 and 1000 units/ml.

There was again very little difference between the controls, Sets I and II. For the first 2 or 3 days larvae in the Clyde water swam a little more strongly than in the E1 water, and in both were more strongly swimming than in the similar waters of Exp. 4, though again neither showed the marked concentration at the surface which had been a feature of the best cultures in previous years. Later there was no difference in swimming vigour to be observed. Structurally there was no difference to be observed between the two sets of larvae throughout the experiment.

The addition of E.D.T.A. made very little difference. When 2 days old the larvae in the waters with E.D.T.A. (Sets III and IV) swam a little more strongly than in the controls, but later they were not quite as vigorous and structurally became a little inferior to the larvae in the same waters without the sequestering agent.

Larvae in the artificial sea water (Set V) were a little inferior in swimming vigour and in structure to the larvae in the natural sea waters (Sets I–IV). They were better in the mixtures (Sets VI and VII), the mixture with Clyde water being slightly the better. The larvae were a little better in the artificial water to which E.D.T.A. had been added (Set VIII) except on the last day when they were worse. The addition of Fe (Set IX) to the artificial sea water made little or no difference to the larvae.

The larvae in the autoclaved Clyde and E1 waters (Sets X and XI) were inferior structurally to those in the controls though for a short time they swam a little more vigorously.

The antibiotics had little or no effect on the larvae.

None of the differences throughout this experiment was anything more than slight.

Discussion

Throughout the 1953 experiments the two sorts of water were closely similar in their effect on the larvae and they never showed as marked a difference as had often, but not always, been a feature of the comparisons made in previous years. None the less the larvae were slightly better formed and a little healthier in the Clyde than in the E1 water, more especially during the early part of the season. Owing to the many variable factors involved, direct comparisons of one year with another are open to more than one interpretation, and cannot very profitably be made. The impression has been gained, however, that the E1 water tested in 1953 was on the whole a little more suitable for *Echinus* larvae, while the Clyde water was a little less so, than on most occasions when used in 1952. Also that in neither were the larvae ever as well formed, or as vigorously swimming, as on the best occasions in earliest years. Their condition was always not very dissimilar from that recorded of the larvae in Exp. III of 1952 (Wilson & Armstrong, 1952, p. 344) where there was also little to choose between the two waters.

We are indebted to Dr Sheina M. Marshall for the information that a tow-net haul made in the Clyde in mid-April 1953 contained forty-seven *Sagitta elegans* and thirteen *Sagitta* too young for certain identification. Mr P. G. Corbin informs us that the standard ½ hour oblique hauls made with the 2 m. stramin ring-trawl, at the Eddystone in March 1953 indicated a sparse plankton characterized by *Sagitta setosa*. In April the hauls both at the Eddystone and E1 (10 miles S.S.W. of the Eddystone) were again small in quantity with only very few *Sagitta*, both *S. setosa* and *S. elegans* being seen. But on 11 May, at E1, when water for our last experiment was collected, the presence in the continuing sparse plankton of 20 *Aglantha rosea* showed an influx of western water, although no *Sagitta* or other indicator species were present. Silicate analyses made by F.A.J.A. also suggest a change in the water mass at E1 by 11 May. This change is paralleled by our results, in so far as such differences as were apparent between larvae reared in waters from E1 and the Clyde were more pronounced in the earlier experiments than in the later.

It was unfortunate that the unsuspected copper in the active carbon used in 1952 gave rise to a misleading result. The sea water passed through that particular sample of carbon must have picked up sufficient copper to have become poisonous to the larvae. The larvae in this year's carbon-treated sea water were similar to those in the untreated controls, but the carbon extracts once again proved poisonous to the developing eggs. These results seem to close what had seemed to be an encouraging approach to the solution of the problem through the use of activated carbon.

The attempts to filter the water free from bacteria at the time of collection were mainly unsuccessful but one quantity of E1 water proved to be bacteria-free when examined just before use. The results with it did not differ in any way from those obtained with the water collected at the same time and merely strained into a carboy. Since the bacterial content of this carboy water was high when tested this result is of interest. It is noteworthy, too, that bacteriostatic concentrations of penicillin and streptomycin were without effect.

The addition of various substances, notably the vitamin B_{12} and the sequestering agent E.D.T.A., did not improve either of the waters: if anything they adversely affected the larvae. Heating the waters generally made them, especially the E1 water, worse as a medium for larval development. In artificial sea water the larvae did moderately well, though not quite as well as in the natural sea waters. It may be possible to use artificial sea water as a standard medium with which to compare natural waters and to which to add any substance the effect of which on the larvae it is desired to test.

Summary

In all comparative tests made in 1953 developing *Echinus* larvae did only slightly better in sea water from the Clyde than in sea water from the English Channel at E1.

The activated carbon used in one of the 1952 experiments contained a significant amount of copper. The experiment was therefore repeated in 1953 with copper-free carbon, and this time the sea water passed through the carbon was not toxic although the carbon extracts were so, as previously found.

Larvae reared in a quantity of E1 water which had been filtered free from bacteria at the time of collection were no different from larvae reared in water collected at the same time in the usual way.

Antibiotics in bacteriostatic concentration had no effect on the waters as media for *Echinus* development.

The addition to the water of extracts of gonads and of filtered coelomic fluid from adult *Echinus* were detrimental to the larvae, as was also filtered sea water in which other larvae had died and decayed.

Additions of the vitamin B_{12} and of the sequestering agent E.D.T.A. had little effect on the larvae, being slightly detrimental.

Heating the waters for shorter or longer periods generally had an adverse effect on larvae reared in them.

Larvae reared in artificial sea water, with or without the addition of iron, were in general inferior to those reared in natural sea waters.

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Printed in Great Britain

THE ATTRACTIVE FACTOR IN THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY

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Largely as a result of experiments made in 1951, sands were classed as attractive, neutral or repellent (Wilson, 1953*a*); but only after the 1952 experiments had been completed did it become reasonably certain that the sands in which the larvae of *Ophelia bicornis* Savigny readily settle carry an attractive factor apparently distinct from a repellent one present in sands in which they will not settle (Wilson, 1953*b*). Whilst the precise natures of the factors were uncertain, it seemed probable that they both derive from organic activity. The 1953 breeding season was devoted to an attempt to obtain more information about the attractive factor.

METHODS

Methods closely followed those used previously. All glassware was cleaned in hot strong sulphuric acid, and similar acid was used in the preparation of the acid-cleaned sands. These were always prepared from Bullhill Bank sand (collected 10 June 1952) generally a few days, and not more than a few weeks before use, being stored, after cleaning, in distilled water in stoppered bottles. Fertilizations and experiments were mostly in a mixture of Berkefeld-filtered sea waters from the Eddystone or E 1 and from the Clyde (obtained by the helpful co-operation of the Millport Marine Station, and Mr E. Latham in particular). Occasionally Clyde water was used alone but never Eddystone or E 1 water alone. Although all fertilizations were not equally successful there was no difficulty in obtaining healthy larvae for all the experiments, as there had been in 1952 on occasions when E 1 water only was used (Wilson, 1953*b*, p. 220). Throughout this paper 'sea water' means Berkefeld-filtered sea water.

The culture medium used was 'Erdschreiber', similar to that described by Gross (1937, p. 754). It was kindly supplied by Dr M. W. Parke from her stock, intended for the culture of diatoms and flagellates.

Sands were always tested in Pyrex crystallizing dishes by the free-choice method (Wilson, 1953*a*, p. 415) and sometimes also by conical-vessel tests (Wilson, 1952, p. 66). The former method distinguishes between attractive and neutral sands, the latter between repellent and neutral sands (Wilson, 1953*b*, p. 210). Very many tests were made, and when time did not allow of the settled larvae being counted the comparative sizes of the settlements were recorded in words. The scale of words used to express number is the same as before, but for convenience it is reprinted here below Table I. All the unmetamorphosed larvae recorded were sticking to sand grains; this is a slight departure from previous practice when larvae seen to swim out of a sand sample were also counted.

Experiments are no longer described in the order in which they were made; the former method of numbering has therefore been discontinued. In this paper all experiments of the same type are grouped under one heading. The results were too numerous for all to be recorded in detail; typical results are given in the tables; others are described in the text. A few inconclusive tests are not mentioned; these did not in any way conflict with past or present findings.

Sands referred to as having been 'soaked', were kept for the period stated in sea water (or other medium) in covered Pyrex crystallizing dishes on a window bench shielded from the sun.

Each experiment was controlled with a small dish, the bottom of which was completely covered with fresh Bullhill Bank sand. This dish is not usually mentioned in the account of an experiment, or shown in the tables, but it was always employed to check the ability of the larvae to metamorphose during the actual period of the experiment. In these control dishes almost every larva was fully metamorphosed after 2 days.

In every free-choice dish, no matter what sands were tested, there were always at the end of the experiment many unmetamorphosed larvae swimming freely, or attached lightly to the surface film. Generally there were also a few sticking lightly to the glass bottom of the dish between the sand heaps. Occasionally one or two metamorphosing or metamorphosed worms were also to be seen on the glass; it was always practically certain that they had crawled out of a sand heap.

THE EXPERIMENTS

Experiments with Filter-Paper

In a preceding paper (Wilson, 1953*b*) it was mentioned (p. 200) that 'some tests based on filtering water in which fresh sands had been shaken gave promising, though imperfect results'. These tests had been included in experiments, carried out in late July 1952, which had been spoilt by the condition of the larvae. The results, so far as they had gone, suggested that whereas acid-cleaned Bullhill Bank sand is made attractive by soaking for some days in sea water in which fresh Bullhill Bank sand had been shaken, it is made even more attractive if the water, after the shaking, is filtered through Whatman No. 42 filter-paper (a fine-textured double acid-washed paper). This surprising result merited further investigation.

Some fresh Bullhill Bank sand, collected (11 June 1953) from the surface of the bank at a place where adult *Ophelia* worms were numerous, was vigorously shaken (12 June 1953) in sea water. After the grains had settled the water was poured off (for convenience such water will in what follows, and in the tables, be referred to as 'B.B. water'), a portion being filtered through a piece of Whatman No. 42 filter-paper. Small quantities of acid-cleaned Bullhill Bank sand were placed in both the filtered and the unfiltered 'B.B. water', and were also placed on the piece of paper used for the filtration. Further quantities were placed on an unused sheet of the paper in sea water, and, for control, in a dish of sea water alone. Portions of these sands were tested with 5-day-old larvae after so soaking for 5 days and some of them again after 26 days. The tests after 5 days were further controlled with acid-cleaned sand straight from storage in distilled water, and the 5-day tests included both dish and conical vessel experiments, those after 26 days dish experiments only.

The results are shown in Table I. They do not confirm the result of last year's imperfect experiment. The acid-cleaned sand which had soaked in unfiltered 'B.B. water' had become definitely more attractive (especially after 26 days) than that soaked in sea water only, but that which had been in the filtered 'B.B. water' seemed to be even slightly less favourable than that from sea water alone. The acid-cleaned sand on the filter-paper through which the 'B.B. water' had been passed seemed to be unaffected (after 5 days), but that on the unused piece of filter-paper had become markedly attractive, inducing heavy settlements in both dish and conical vessel experiments. This result being the reverse of what had been expected it may be as well to note that there is no possibility of the two papers, or the sands from them, having been interchanged or confused during the experiment. It should be noted, however, that the paper used for the filtration of the 'B.B. water' unfolded itself when placed in a dish of water and much of the sand sprinkled over it may not have been on that part of the paper which had picked up most of the particles from the water filtered through it.

At the time of the second test, after 26 days, the acid-cleaned sands from both filtered and unfiltered 'B.B. waters' had minute growths, apparently algal, on the grains and contained some ciliates. These were most numerous in the sand from the unfiltered water, and this sand was, in addition, a little silty. The control sand, from sea water only, appeared clean. These observations were made with a dissecting binocular at a magnification not exceeding × 100.

Some further experiments were made with filtered (No. 42 paper) and unfiltered 'B.B. water', and with the intention of obtaining improved results culture medium was added to equal quantities of the 'B.B. water' and this was controlled by diluting other portions of the 'B.B. water' with equal amounts of sea water, as well as using normal sea-water controls. The results of these more elaborate experiments were less definite than the one just described. The culture medium itself had little or no effect on the acid-cleaned sands soaked in it, neither did it appear to increase the potency of the 'B.B.

water' to which it was added. Dilution of the 'B.B. water' may have decreased its powers of making the acid-cleaned sand attractive, at least for the relatively short periods (not exceeding 16 days) the sand was soaked in it. The results, while not showing very marked differences between the variously soaked sands, are in general agreement with those of the earlier experiment. So far as they go they do indicate that acid-cleaned sand is made attractive by soaking in unfiltered 'B.B. water', but is little affected if the 'B.B. water' first be filtered through No. 42 paper.

The unexpected result in the first experiment (Table I) where an acid-cleaned sand became attractive by contact, in sea water, with unused Whatman No. 42 filter-paper led to a series of experiments to investigate the possibility that the filter-paper contained some substance which became adsorbed on the grains or which encouraged bacterial or other growths attractive to the larvae.

It is not proposed to consider all these experiments in detail. Some of them were negative, but the majority were slightly in favour of the filter-paper having some such favourable influence. The best result was obtained by soaking acid-cleaned sand in the first small quantity of sea water passed through a new piece of paper, especially (Table II) after soaking for several days. After much sea water had been passed through the paper the last quantity of water collected did not have as marked an effect. Never again was a really good settlement obtained with acid-cleaned sand allowed to lie on, or between, unused filter-papers in sea water. Indefinite results were obtained with concentrated extracts of several filter-papers, and with filter-papers washed in strong hydrochloric acid followed by distilled water. All the papers came from the same box and care was taken to avoid contamination during handling. The results are quite inconclusive and are of value only in indicating that results obtained with acid-cleaned sands soaked in filtered 'B.B. water' will either not be influenced at all, or if they are they will be in the direction of attractiveness. Thus the results with filtered 'B.B. water' already discussed can at least be accepted provisionally. It may be too, that the explanation of last year's imperfect experiment, which led to this investigation, can be explained by the favourable action of the particular piece of filter-paper then used.

An Experiment with Sea Water in which Fresh Sand from the Salthouse Lake was Shaken

From one of last year's experiments (Wilson, 1953*b*, Expt. 57B) it appeared that sea water in which fresh sand from the Salthouse Lake (Station II) was shaken was capable, like 'B.B. water' of making attractive an acid-cleaned sand soaked in it. To check this result a similar experiment was made this year. After washing away, with sea water, all the easily removable silt a quantity of surface sand from the Salthouse Lake (Station II) was, the day after collection, vigorously shaken in sea water. After settlement of the grains the sea water, now rather dirty, was poured off. It was divided into two portions, to one an equal volume of clean sea water was added, to the other an equal volume of culture medium. To these and to controls, consisting of sea water only and sea water plus an equal volume of culture medium, acid-cleaned sand from storage in distilled water was added. The sand was soaked for 7 days and then tested with 5-day-old larvae, in a free-choice dish and in conical vessels. After 2 days the results were not very definite (see Table III) but it was noted that the larvae used in this experiment swam unusually strongly, keeping mainly to the surface of the water. It was therefore decided to continue the experiment for another 3 days, and the sands from the free-choice dish were carefully put back again and some more larvae from the original culture (now 7 days old) were added. The conical vessel tests were not repeated. After a further 3 days in the dark the free-choice dish sands were again examined, with the result shown in Table III. There seems no doubt that the acid-cleaned sand had been improved by soaking in the 'S.L. water' and that the addition of culture medium, as had been found in several other experiments, has little or no effect on acid-cleaned sands soaked in it.

Acid-cleaned Sands Soaked in Sea Water in the Presence of Fresh Natural Sands

The experiences with 'B.B. water' and 'S.L. water' led to another type of experiment in which an acid-cleaned sand was placed at one side of a dish with freshly collected Bullhill Bank or Salthouse Lake sand at the other. Such acid-cleaned sands when tested after only a few days showed no difference from the controls kept in sea water only, but after several weeks they markedly improved in attractiveness, but then so too did the controls, though usually not to quite the same extent. A series of tests is recorded in Table IV from which it appears that the fresh Salthouse Lake sand had an even more favourable influence on the acid-cleaned sand kept with it than had Bullhill Bank sand itself. This, however, was not so in the experiment recorded in Table V. It is unfortunate that the experiment being lengthier than originally anticipated the control acid-cleaned sand kept in sea water had all been used up before the last tests were made.

In another series of tests (see Table V) the fresh sands occupied most of the bottom of the dish, the acid-cleaned sand being placed in cleared areas in the middle. The cleaned sands were thus completely surrounded by the fresh sands. A mixture of equal parts of sea water and culture medium was used instead of sea water only. Fresh sand for a depth of 12–18 in. on the Bullhill Bank was tried as well as sand from the surface. The Salthouse Lake sand was, as usual, from the surface layer. The results, while not very definite, point to a slight improvement of the acid-cleaned sand surrounded by the fresh Bullhill Bank surface sand and little or no improvement by the deep sand or by the sand from the Salthouse Lake. It is possible that the soaking times were too short for definite results. Moreover, the larvae used on 2 August 1953 (and this applies also to the previous experiment) were noticeably less ready to settle than usual for their age. This is deduced from their tendency to swim vigorously up against the surface film and from their behaviour in control dishes (not recorded in the tables) plentifully strewn with fresh Bullhill Bank sand. As has been pointed out before (Wilson, 1953*b*, p. 221), it is usual for some cultures of larvae to show less readiness to metamorphose than others of the same age.

Acid-cleaned Sands Soaked in Sea Water, in Distilled Water and in Culture Medium

Towards the end of the 1953 series of experiments it was found that acid-cleaned sands which had been soaked in sea water as controls for earlier experiments had become markedly attractive to the larvae. An acid-cleaned sand soaked for a few days in sea water had often induced a few more larvae to settle in it than had the same sand stored in distilled water and tested with it in the same free-choice dish. However, the increase in attractiveness had almost always been very small. With acid-cleaned sands in sea water for a varying number of days (from 1 to 26) there were only two occasions out of twenty-three when 5-day-old larvae settled in appreciable numbers (several metamorphosed and several metamorphosing) and on those two occasions (one after 5 days of soaking and one after 10) there were no tests of sands from distilled water storage with which to compare. It is possible that the larvae used in those particular experiments were more ready to settle than usual.

There were three tests with 6-day-old larvae with sands soaked for 20, 22 (see Table VII) and 25 days, and these all gave good settlements, but no comparative tests with acid-cleaned sands from distilled water were made. It must be remembered that 6-day-old larvae usually settle more readily than do 5-day-old.

Of three tests with acid-cleaned sand soaked for 37, 40 and 43 days, respectively, all induced good to heavy settlements while there were only light settlements in sand from distilled water storage tested with them. Of these three results one is recorded in Table IV (virtually with 6-day-old larvae), the other two in Table VI which shows the results of four tests of an acid-cleaned sand kept in sea water over a period of nearly 6 weeks. In the last test, on 25 July 1953, the larvae were in the free-choice dish for 3 days instead of the usual 2.

It will be realized that the experiments were not planned as they are here presented. This explains the lack of uniformity with regard to the age of the larvae used for testing and the occasional absence of control sands from distilled water. It was not until late in the season that there was any suspicion that soaking acid-cleaned sands in sea water for long periods improved them, and it was then too late to arrange further experiments. None the less, from the data just given, it seems impossible to avoid the conclusion that soaking in sea water has this favourable effect.

Reference has frequently been made to using acid-cleaned sand stored in distilled water as a control. During the course of the 1953 experiments such sand was tested sixteen times in free-choice dishes. Only on one occasion (recorded in Table VI) was there even a moderate settlement; in all other instances there was either no settlement or a very small one. Acid-cleaned Bullhill Bank sand stored for over a year in distilled water was tested with 6-day-old larvae in a free-choice dish with sand stored for 50 days only. In neither, after 2 days, were any metamorphosed worms to be found, and only a few or very few metamorphosing ones, and about the same number unmetamorphosed. Thus acid-cleaned sand kept in distilled water does not increase in attractiveness, as it does when kept in sea water.

In several experiments culture medium was added to the sea water in which acid-cleaned sand was soaking, with the intention of encouraging the growth of autotrophic organisms already present in fresh sands. The effect of culture medium added to sea water only had therefore to be determined. In Tables III, V and VII comparisons between sands from sea water only and from sea water to which culture medium was added have already been given, and there were a number of others. Of eleven distinct tests there was no difference in four; in the remaining seven the sand from the culture medium was either slightly more attractive (three tests) or slightly more repellent (four tests, the most positive in the repellent direction being recorded in Table VII) than sand from sea water only. It may thus be concluded that for the relatively short periods of soaking employed (not more than 22 days) the addition of culture medium to plain sea water has no appreciable effect.

Experiments with Diatom and Flagellate Cultures

The possibility that the attractiveness of Bullhill Bank natural sand is due, partly at least, to the presence on the grains of living micro-organisms led to experiments in which acid-cleaned sand was kept for 2–3 weeks with living diatoms and flagellates. A mixture of several species of each (kindly provided by Dr M. W. Parke from the stock cultures kept at the Laboratory) was used for the first experiment, the results of which are recorded in Table VII. Complete counts were made of the larvae settled in the two controls (tests 1 and 2), but time did not allow of a complete count of the much larger number of larvae settled in the sand with the diatoms and flagellates (test 3), and after as many as possible had been removed and counted it was obvious that at least as many again were left in the sand. It should be noted that the sand was rinsed in plain sea water before testing and many diatoms and flagellates were then washed away. The sand grains were thus left relatively clean, but numbers of minute diatoms and flagellates could be seen on the grains while the count of larvae was being made. Conical vessel tests (not shown in the table) of the same sands, run concurrently, gave fairly heavy and very similar settlements for all three sands, from which it should probably be inferred that the increased attractiveness of the sand from the diatom and flagellate culture was actually only slight. This seems to be confirmed by a later experiment (Table VIII) in which mixed diatoms and mixed flagellates were used separately as well as together. It was only in the sands which had been in the presence of flagellates that any noticeable increase in settlement was obtained and even here none had fully metamorphosed after 3 days in the free-choice dish (no conical vessel tests were made). In this experiment numerous flagellates were stuck to the sand grains but the diatoms seem to have been lost from the sand during the rinse in sea water immediately before testing.

Taking the two experiments together it seems reasonable to conclude that the presence on the acid-cleaned grains of flagellates, or possibly bacteria from their particular cultures, has some slight attraction for the larvae.

An attempt was made to culture organisms from fresh Bullhill Bank sand and transfer these to acid-cleaned sands. Diatoms and some other organisms multiplied in culture medium but the acid-cleaned sand kept with them was only slightly improved after 15 days. The grains were covered with adherent diatoms, singly and in clusters, in a manner not seen in fresh Bullhill Bank sand. There may have been too many of them.

Experiments with Organic Extracts

An experiment with acid-cleaned sand kept for half an hour in an extract of *Ulva*, and for the same period in starch dextrine gave almost negative results. The *Ulva* extract had no effect, but the sand with the starch was apparently slightly improved compared with the same sand from distilled water. However, a later test with sand kept in starch dextrine for 28 h was completely negative.

Acid-cleaned Sand kept with Adult Ophelia bicornis

In a previous experiment (Wilson, 1953*a*, Expt. 51) some washed Salthouse Lake sand kept with adult *Ophelia* worms for several days had been made even less attractive than before. It was considered desirable to repeat this experiment with acid-cleaned Bullhill Bank sand. Accordingly, a few spent worms were kept in a clean dish for 2 or 3 days until they had emptied their guts of all sand grains. They were then transferred to another dish with a fair quantity of acid-cleaned Bullhill Bank sand. It was noticeable that they would not burrow into this sand (as they normally do into fresh sand), but lay on the top. After 4 days a little of this sand was tested, both by free-choice dish and conical vessel tests, against sand from sea water only. It was found to attract slightly fewer larvae than the latter, but in both the settlements were small.

A similar result was obtained with more of the same sand after 16 days with the adult *Ophelia* worms. The presence of the adult worms had not improved the sand, neither had they markedly worsened it.

Tests for Gregarious Settlement

It has been shown that the larvae of the oyster (Cole & Knight-Jones, 1949), of the acorn barnacle *Elminius modestus* (Knight-Jones & Stephenson, 1950) and of the polychaete *Spirorbis borealis* (Knight-Jones, 1951) when ready to settle are attracted by the presence of newly metamorphosed young of their own species. There was therefore a distinct possibility that the larvae of *Ophelia bicornis* might show a preference for settling in sand in which others of their kind were metamorphosing or had recently metamorphosed. In order to test for this two experiments were arranged in which 5-day-old larvae were given a choice between acid-cleaned sand containing metamorphosing and recently metamorphosed larvae from an earlier fertilization and similar sand without. In order to distinguish the younger larvae from the older the former were lightly stained with methylene blue. The ability of the stained larvae to metamorphose was concurrently tested in another dish containing fresh Bullhill Bank sand, in this they settled and metamorphosed normally and as readily as did larvae from the same fertilization not stained and separately tested.

The result of the first of these experiments is given in Table IX from which it will be seen that unfortunately three of the unstained older and recently metamorphosed young worms had during the course of the experiment crawled into the pile of sand which was not supposed to contain them. There were, of course, very many more of these young worms in the other pile, the sand of which was spread by their activities until it occupied a greater area than it had done originally. On the clean glass bottom of the dish, particularly in the neighbourhood of this pile, were a number of metamorphosing and metamorphosed larvae, both stained and unstained, which had apparently crawled out of the sand. These were not counted, and the figures given for this pile in the Table are therefore lower than they should be, but only by a small fraction of the total possible. A curious feature of the figures for the stained younger larvae is that almost all found in the sand (there were many still swimming freely in the dish or lightly attached to the surface film of the water) were fully metamorphosed. This is unusual for this type of dish experiment, where newcomers normally settle in an attractive sand at any time throughout the duration. It seems that here settlement must have taken place mainly during the first or second day and very little afterwards, and it is not clear why this should be. The number settled in the sand is higher than what would normally be expected in a neutral acid-cleaned sand, but there is no significant difference between the pile with the three older unstained worms and the pile with the 114. Of course, if any stimulating soluble substance is given off during metamorphosis, or for a short while afterwards, it would quickly diffuse throughout the dish and affect both sands alike. That this hypothetical substance, the actual existence of which is doubtful, did not of itself, without the presence of sand, bring about metamorphosis is obvious from the large number of younger stained larvae still swimming freely in the dish, or attached singly or in clusters, unmetamorphosed, to the surface film. The correct interpretation of these results is thus not clear, but they do not seem to offer much support for the existence of gregarious settlement in this species.

The second and later experiment, the results of which are recorded in Table X, are in general agreement with the conclusion from the earlier one. Conducted on similar lines, with 5-day-old larvae stained before use, it differed only in using acid-cleaned sand from distilled water as the control and fewer settling older and unstained larvae in the test sand. Some of these unstained larvae must have migrated to the control sand early in the experiment and quite a number were found, unmetamorphosed, on the surface film with stained larvae when the dish was examined immediately after removal from the dark box in which it had been kept for 3 days. A fair number of metamorphosed young worms, unstained, and one or two stained were crawling on the glass bottom of the dish, especially around both piles of sand. In comparison with the first experiment it is noticeable that only a small number of stained larvae settled in either pile.

Experiments with an Almost Pure Charcoal

In many previous experiments forms of activated charcoal have produced heavy settlements, but it has never been quite certain whether it was the carbon which attracted the larvae or some impurity such as copper (Wilson, 1953*b*, p. 224). I am indebted to Mr F. A. J. Armstrong for making from 'AnalaR' sucrose a charcoal almost totally free from impurity (analysis for copper, by F.A.J.A. gave less than 0·2 parts/million). That this charcoal had some absorptive properties was demonstrated by its power to decolorize weak dye solutions. The sucrose charcoal was, for testing, mixed with an acid-cleaned sand; the results are recorded in Table XI (two separate tests with different batches of larvae). The larvae were undoubtedly strongly attracted by it, and if the numbers settling seem fewer than in some earlier experiments with other forms of charcoal this is to be explained by failure to obtain an intimate mixture of the sucrose charcoal with the sand. This charcoal was much coarser than that manufactured for decolorizing purposes, so often used previously, and did not adhere to the grains at all. The particles were angular and the mixtures contained much more sand than charcoal.

From these results it is probably permissible to conclude that the carbon of the charcoal rather than the impurities attracts the larvae, but whether for its absorptive property or for some other reason is still unknown.

Other Experiments

The possibility that the minute amount of copper contained in activated charcoal might stimulate the larvae to metamorphose (Wilson, 1953*b*, p. 224) led to an experiment in which acid-cleaned sand was soaked for an hour in 100 μg Cu/100 ml. sea water. The sand was then well washed in filtered sea water and tested in a free-choice dish with similar sand not so soaked as a control. After 2 days the control sand contained one or two metamorphosing larvae and two or three unmetamorphosed ones, while the sand from the copper solution contained only a large number of unmetamorphosed larvae, many of them dead. A likely explanation is that larvae exploring the sand were quickly weakened by the copper and, unable to swim away, were eventually killed by it.

The 1952 experiments (Wilson, 1953*b*) included several tests in which neutral sands (contained in bolting-silk envelopes) buried in fresh sands acquired an attractive factor from fresh Bullhill Bank sand but not from fresh Salthouse Lake (Station II) sand. It has also been shown that heating Bullhill Bank sand in water to about 100° C (previously referred to as 'normal sterilization') destroys the attractive factor, but no check had been made in which a neutral sand, inside a bolting-silk envelope, had been buried in sand so heated. Thus among the present experiments were included tests of an acid-cleaned sand, contained within bolting-silk envelopes, buried in heated Bullhill Bank sand and in heated Salthouse Lake (Station II) sand, in sea water for 8 days. The buried sands were then presented to larvae in a free-choice dish and in conical vessels, using as control a similar sand kept for 8 days in sea water only. The sand exposed to the heated Bullhill Bank sand induced light settlements almost identical with those in the control, but that exposed to the heated Salthouse Lake sand induced smaller settlements, suggesting that it had become a little repellent.

Discussion

There have been few methods by which a neutral sand has been transformed into an attractive one. They have included the use of charcoal, a substance unlikely to be present in the Exe sandbanks, and extracts of filter-paper which gave the curious results already discussed. Almost the only other methods have all employed some form of soaking the neutral sand in sea water under conditions likely to encourage growths of micro-organisms too small to be readily visible during normal examination of the sands. It will therefore be well to consider what evidence there is from these experiments that such organic growths do indeed form on clean sand grains, immersed in sea water, and what evidence that the larvae react to their presence.

The first fact to note is that acid-cleaned sand stored in distilled water, in stoppered bottles, even for over a year, does not increase in attractiveness.

Distilled water, devoid of nutrients, does not encourage growths. On the other hand, it is improbable that anything kept in even Berkefeld-filtered sea water, contained in a perfectly clean dish covered only by a loose glass plate, could remain for long free from bacteria, etc. Such sea water must almost always contain some nutrient material sufficient for small growths to take place. After several weeks small organisms, motile and non-motile, have always been seen in such dishes, and a scratch on the bottom with a needle has generally revealed a very thin film of slime.

I am indebted to Dr C. H. Jellard (Public Health Laboratory Service) for endeavouring to demonstrate, by normal bacteriological techniques, that acid-cleaned sand does become coated by bacteria, etc., after days or weeks in filtered sea water. Unfortunately the irregularities, natural scratches, colourings and other markings on the sand grains made this an almost impossible task. In substitution for the sand grains we immersed acid-cleaned cover glasses in Berkefeld-filtered sea water for various periods, and it was then easy to see that they indeed became covered by bacteria and other organisms and that these increased in abundance for a time, never becoming excessively numerous. The control cover-glasses, kept in distilled water in stoppered bottles, remained clean, as was expected.

That the organisms which develop in dishes of filtered sea water are not always the same species is apparent when such dishes are compared after several weeks. Some will then contain minute diatoms not present in others, or rounded unidentified objects or flocculent growths absent from other dishes on the same bench close by. Occasionally minute ciliates or flagellates are seen. Acid-cleaned sand kept in these dishes for several weeks or even months looks clean until close inspection reveals that there are on the grains small numbers of the organisms which are to be seen more easily on the glass of the dish. It is likely that some species are more acceptable to the larvae than are others. This is to some extent indicated by the experiments with diatoms and flagellates from culture; the flagellates (or unseen organisms introduced with them) having some slight attraction for the larvae, the diatoms seemingly none at all, even diatoms cultured from Bullhill Bank itself. Differences in the kinds of organisms growing on the acid-cleaned sands after days or weeks in sea water could explain some of the small variations in intensity of settlement in sands from different dishes, or in sand from the same dish tested at different times. But on the whole it remains true that, within the period of time covered by the 1953 experiments, the longer the acid-cleaned sands were soaked in sea water, the more they increased in attractiveness.

The fact that acid-cleaned sand soaked in sea water in which fresh Bullhill Bank or Salthouse Lake sands had been shaken becomes more attractive than the same sand kept for an equal time in sea water only is very suggestive of living organisms. It may be assumed that many organisms are washed off the natural sands by the shaking and subsequently adhere to and grow on the acid-cleaned grains.[1] Moreover, these are likely to be the species that are the natural food of the young worms. Even the presence of the fresh sands in the dish with the acid-cleaned sand eventually imparts to the latter a little more attractiveness than soaking for a like period in sea water alone. The addition, in the 1952 experiments (Wilson, 1953*b*), of a few grains of fresh sand, from either the Bullhill Bank or the Salthouse Lake, imparted a degree of attractiveness to acid-cleaned sands, but more markedly so after several days at room temperature during which organisms introduced on the fresh grains would have had time to multiply and spread. This happened both in the light and in the dark, and although other explanations are possible this suggestion that living organisms, multiplied after a lapse of time, were responsible for the increased attractiveness is at least reasonable. Some similar inoculation experiments, in 1952, using weak media instead of sea water only, and at a raised temperature in a warm dark oven, had similar though less definite results.

The burying of a neutral sand, enclosed within a bolting-silk envelope, in fresh Bullhill Bank sand has been shown to be a sure way of making that sand attractive (Wilson, 1953*b*). But if the Bullhill Bank sand is first heated to about 100° C in water no such increase of attractiveness is apparent. This again can be explained on the assumption that in the first instance living organisms pass through the bolting silk to mingle with the neutral sand, as indeed they were observed to have done, whereas in the second instance there would be no living organisms in the sand after heating. In all earlier experiments treatments of fresh Bullhill Bank sand which would have killed organisms living in it have always reduced its attractiveness to *Ophelia* larvae (for a summary of these treatments see Wilson, 1953*a*, pp. 423–4).

Occasionally, after treating a neutral sand by one or other of the methods by which micro-organisms would be encouraged to grow on it the sand has induced a settlement about half as large as that obtained in fresh Bullhill Bank sand, presented to the larvae under the same conditions. This is a decided advance on anything achieved during earlier breeding seasons when almost the only known method of ensuring, especially under free-choice conditions, a heavy settlement in sands other than fresh Bullhill Bank sand was to use activated charcoal, the attractiveness of which for the larvae is still unexplained.

The solution of the main problem, that of explaining how *O. bicornis* larvae recognize their own natural sand, now seems to be within reach. From the results of previous work it can be accepted that suitable grade is one of the factors influencing the larvae in their choice of a sand in which to settle. Cleanliness is another. But this cleanliness is only relative, for a sand of suitable grade, if perfectly free from all organic matter, is neutral in the sense of neither encouraging nor discouraging larvae to settle. Only when the relatively clean grains carry living micro-organisms, or their organic products, are they really attractive to larvae in the exploratory phase prior to settlement. It seems most likely that these micro-organisms must belong to a limited, though perhaps fairly extensive range of types and species, and that there is an optimum abundance just as there seems to be an optimum size range of sand grains. If the two optima coincide then there will be produced the best possible conditions for settlement.

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[1] Filtration of the 'B.B. water' through No. 42 filter-paper generally reduced its efficiency in making attractive acid-cleaned sand soaked in it (see pp. 363–4) but in view of the inconsistent effects of filter-paper these results are not advanced in support of the present thesis.

An explanation such as that just outlined would bring *O. bicornis* larvae more or less into line with the larvae of some sedentary animals which do not settle on solid surfaces until after the surfaces have become coated with bacteria or other organisms or with the slime films produced by them (for references and a short discussion see Wilson, 1952, pp. 52–3), although these are probably not sought for as food. It would also fit the many facts now known concerning the settlement reactions of *Ophelia* larvae.

Attraction is, however, only half the picture. During 1953 no attention was paid to a repellent factor which seems to co-exist with an attractive one on the Salthouse Lake sands in which adult *Ophelia* does not live. It has previously been suggested that the larvae may dislike some kinds of dead organic matter on the sand grains, or the presence of too many micro-organisms, or the wrong kinds. It is probable that variations in the quantity and quality of the organic matter, living or dead, on and among the sand induces corresponding variations in the intensity of the settlement of *Ophelia* larvae coming into contact with them.

SUMMARY

During the 1953 breeding season attention was paid to ways in which neutral acid-cleaned sand may be made attractive to the larvae of *Ophelia bicornis*. It was found that one of the most effective methods is to soak the sand for a long period in sea water, during which time there is evidence that the grains acquire a coating of living micro-organisms. Similar soaking in water in which fresh sands have been shaken, or in the presence of fresh sands in the same dish, are even more effective ways of rendering the neutral sand attractive. It is concluded that the presence on the grains of living micro-organisms, or products of their activity, in not too great an abundance, makes the grains attractive to the larvae.

Acid-cleaned sand soaked for long periods in distilled water is not made attractive to the larvae, neither does it acquire a coating of micro-organisms. This was demonstrated by substituting acid-cleaned cover-glasses for sand, and comparing the results of keeping them in distilled water and in sea water.

An acid-cleaned sand enclosed within a bolting-silk envelope and buried, in sea water, in Bullhill Bank sand, previously heated to about 100° C, does

not become attractive, as it does when the Bullhill Bank sand is fresh and unheated. The difference is regarded as being due to the absence of living organisms in the pre-heated sand.

Diatoms alone are ineffective in making a neutral sand attractive to the larvae, but the presence of flagellates appears to impart some slight attraction.

The addition of culture medium (Erdschreiber) to the sea water in which the sands are soaked is without significant effect. This type of culture medium may not be a suitable nutrient fluid for the kinds of organisms the larvae like.

It is shown that a very pure form of charcoal, made from sucrose, is as effective in inducing larvae to settle as were impure forms in earlier experiments. It is concluded that it is the carbon itself, and not contained impurities, which is attractive, although why this should be is still an unsolved problem.

It was found that acid-cleaned sand is sometimes made attractive by contact in sea water with Whatman No. 42 filter-paper, or in extracts from it. The results are, however, erratic and their significance obscure.

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TABLE I

			Acid-cleaned Bullhill Bank sand soaked in					
Tests beginning	Days of soaking		(1) Sea water	(2) 'B.B. water'	(3) 'B.B. water' through filter-paper	(4) Sea water on filter-paper used for no. 3	(5) Sea water on unused filter-paper	(6) Distilled water
			Settlements after 2 days in free-choice dish					
17. vi. 53	5	Metd	2	3	1	None	35	1
		Meting	3	4	3	1	16	1
		Unmet.	7	12	12	7	12	5
			Settlements after 2 days in conical vessels					
17. vi. 53	5	Metd	2 or 3	Several	2 or 3	1 or 2	Many	1 or 2
		Meting	Fair number	Good number	Several	Several	Many	Several
		Unmet.	Fair number	Many	Fair number	Several	Several	Fair number
			Settlements after 2 days in free-choice dish					
8. vii. 53	26	Metd	1 or 2	Good number	None	—	—	—
		Meting	Few	Several	None	—	—	—
		Unmet.	Several	Several	Several	—	—	—

Metd = metamorphosed; meting = metamorphosing; unmet. = unmetamorphosed. 'B.B. water'—see text, p. 363.

Scale of words used to express number in Tables I–XI:

Very few (sometimes also expressed in numbers 1–4)
Few
Several
Fair number
Good number
Many
Very many
Multitude

TABLE II

	Set A. Acid-cleaned Bullhill Bank sand soaked for 2 h in				
	(1) Sea water	(2) Sea water and between 2 pieces of filter-paper	(3) Sea water and between 2 pieces of filter-paper previously washed with HCl	(4) First small volume of sea water passed through one filter-paper	(5) Small volume of sea water passed through the filter-paper used for (4) after previous passage of much sea water
Metd	None	None	None	None	None
Meting	Several	Several	Several	Several	Several
Unmet.	Several	Several	Several	Fair number	Several
	Set B. Acid-cleaned Bullhill Bank sand soaked for 5 days as above				
Metd	Several	Few	Several	Many	Fair number
Meting	Several	Several	Fair number	Many	Fair number
Unmet.	Several	Several	Several	Fair number	Several

The larvae used for both sets were from the same culture and were 5 days old. A single free-choice dish was used for testing each set and the settlements were those obtained after 2 days. Different filter-papers were used for each set.

TABLE III

	Acid-cleaned Bullhill Bank sand soaked for 7 days in			
	(1) Sea water	(2) Sea water plus an equal volume of culture medium	(3) 'S.L. water' plus an equal volume of sea water	(4) 'S.L. water' plus an equal volume of culture medium
	Settlements after 2 days in free-choice dish			
Metd	None	None	1	None
Meting	None	None	1	None
Unmet.	Very few	Very few	Few	Few
	Settlements after 2 days in conical vessels			
Metd	1 or 2	None	Several	Few
Meting	Very few	Very few	Fair number	Good number
Unmet.	Good number	Good number	Several	Several
	Settlements after 3 further days in free-choice dish			
Metd	3	5	24	29
Meting	3	3	6	14
Unmet.	5	5	5	12

TABLE IV

			Acid-cleaned Bullhill Bank sand soaked in			
Tests beginning	Days of soaking		(1) Sea water	(2) Sea water with fresh Bullhill Bank sand	(3) Sea water with fresh Salthouse Lake sand	(4) Distilled water
			Settlements after 2 days in free-choice dish (except for tests beginning 25. vii. 53 which were after 3 days)			
17. vi. 53	5	Metd	1 or 2	None	None	1
		Meting	1 or 2	1 or 2	2 or 3	1
		Unmet.	Several	Several	Several	5
8. vii. 53	26	Metd	1 or 2	—	—	—
		Meting	Few	—	—	—
		Unmet.	Several	—	—	—
25. vii. 53	43	Metd	Fair number	Good number	Many	Very few
		Meting	Very few	Several	Many	Very few
		Unmet.	Very few	Fair number	Fair number	Very few
2. viii. 53	51	Metd	—	10	47	None
		Meting	—	7	16	None
		Unmet.	—	5	8	Very few

TABLE V

Tests beginning	Days of soaking		Acid-cleaned Bullhill Bank sand soaked in				
			(1) Sea water	(2) Sea water plus an equal volume of culture medium	(3) Surrounded by fresh Bullhill Bank surface sand, in sea water plus culture medium as (2)	(4) Surrounded by fresh Bullhill Bank deep sand in sea water plus culture medium as (2)	(5) Surrounded by fresh Salthouse Lake surface sand, in sea water plus culture medium as (2)
			Settlements after 2 days in free-choice dish				
22. vii. 53	9	Metd	1 or 2	Few	Several	Very few	Very few
		Meting	Few	Several	Several	Few	Several
		Unmet.	Several	Several	Several	Fair number	Several
2. viii. 53	19	Metd	Few	1 or 2	Several	Very few	Few
		Meting	Very few	Very few	Very few	Few	Very few
		Unmet.	Very few	Few	Very few	Several	Few

TABLE VI

Tests beginning	Days of soaking		Acid-cleaned Bullhill Bank sand soaked in	
			(1) Sea water	(2) Distilled water
			Settlements after 2 days in free-choice dish (except for tests beginning 25. vii. 53 which were after 3 days)	
20. vi. 53	5	Metd	None	—
		Meting	2 or 3	—
		Unmet.	Fair number	—
23. vi. 53	8	Metd	None	—
		Meting	1 or 2	—
		Unmet.	Several	—
22. vii. 53	37	Metd	Many	Few
		Meting	Many	Several
		Unmet.	Several	Fair number
25. vii. 53	40	Metd	Good number	Very few
		Meting	Few	Very few
		Unmet.	Several	Very few

TABLE VII

	Acid-cleaned Bullhill Band sand 22 days in			
	(1) Sea water	(2) Culture medium	(3) Culture medium containing diatoms and flagellates	
Settlements obtained with 6-day-old larvae after 2 days in free-choice dish				
Metd	22	16	(31)	All × 2 at least
Meting	35	21	(44)	
Unmet.	76	45	(65)	

TABLE VIII

	Acid-cleaned Bullhill Bank sand 15 days in				
	(1) Sea water	(2) Sea water plus an equal volume of culture medium	(3) Medium as (2) containing diatoms	(4) Medium as (2) containing flagellates	(5) Medium as (2) containing diatoms and flagellates
	Settlements obtained with 5-day-old larvae after 3 days in free-choice dish				
Metd	None	None	None	None	None
Meting	Very few	Very few	Very few	Fair number	Fair number
Unmet.	Few	Few	Few	Many	Many

TABLE IX

	Acid-cleaned Bullhill Bank sand 5 days in			
	(1) Sea water		(2) Sea water with larvae (unstained) 6 days old at the beginning of the period	
	Stained larvae	Unstained larvae	Stained larvae	Unstained larvae
	Settlements after 3 days in free-choice dish			
Metd	23	3	28	114
Meting	2	None	2	1
Unmet.	6	None	10	1

TABLE X

	Acid-cleaned Bullhill Bank sand			
	(1) Stored in distilled water		(2) In sea water for 3 days with larvae (unstained) 5 days old at the beginning of the period	
	Stained larvae	Unstained larvae	Stained larvae	Unstained larvae
	Settlements after 3 days in free-choice dish			
Metd	None	Very few	None	Fair number
Meting	Very few	Several	Very few	Several
Unmet.	Several	Few	Several	Few

TABLE XI

	Acid-cleaned Bullhill Bank sand	
	(1) From storage in distilled water	(2) Sand as (1) mixed with charcoal made from sucrose
Settlements obtained with 5-day-old larvae after 2 days		
A 1 in free-choice dish		
Metd	None	Fair number
Meting	2 or 3	Fair number
Unmet.	Several	Fair number
A 2 in conical vessels		
Metd	1 or 2	Fair number
Meting	Several	Fair number
Unmet.	Fair number	Fair number
B in free-choice dish		
Metd	1	12
Meting	4	14
Unmet.	13	63

A 1 and A 2 ran concurrently and used larvae from the same fertilization. B was at a later date with larvae from a different fertilization.

J. Mar. biol. Ass. U.K. (1954) 33, 381–392
Printed in Great Britain

PHOSPHORUS AND SILICON IN SEA WATER OFF PLYMOUTH DURING THE YEARS 1950 TO 1953

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(Text-figs. 1–3)

The analyses reported here refer to water from International Hydrographic Station E1 (lat. 50° 02′ N., long. 4° 22′ W.). They extend the sequence begun in 1923 when sufficiently sensitive chemical methods were first applied at this station (Atkins, 1923*a*), and reported thereafter in a series of papers from this Laboratory (Atkins, 1923*a*, *b*, 1924, 1926*a*, *b*, 1928, 1930, 1953; Cooper, 1933*a*, *b*, 1937, 1938; Harvey, 1948, 1950; Armstrong, 1951; Armstrong & Harvey, 1950). This is a factual report and interpretation and comparison with earlier years are deferred.

Since early in 1948 analyses have been made at roughly monthly intervals by the methods listed below, for phosphate and 'total' phosphorus, and, since early in 1950, for silicate also.

I am obliged to Lt.-Cdr. C. A. Hoodless and the crew of R.V. *Sabella*, and to Capt. W. Creese and the crew of M.F.V. *Sula* for assistance at sea. I am also indebted to Dr L. H. N. Cooper for sometimes taking samples at sea, and to him and to Dr H. W. Harvey for their encouraging interest in this work.

METHODS

Samples were taken at approximately monthly intervals, as nearly as the weather allowed. A Nansen-Pettersson insulated water-bottle was used, except occasionally in rough weather, when a wooden bucket was used for taking surface samples. Samples for silicate determinations were kept in polyethylene bottles. Analyses were completed within 48 h of sampling.

Phosphate and 'total phosphorus' were determined by the absorptiometric methods of Harvey (Harvey, 1948; Armstrong, 1949). The results are inherently free of 'salt-error', and are given in microgram-atoms of phosphorus per litre. The factor for conversion to milligrams P per cubic metre is 30·98, and for milligrams P_2O_5 per cubic metre 70·98. Phosphate results include arsenate, 1 μg atom of As as arsenate being determined as 1 μg atom of P as phosphate. Arsenic in sea water at Station E1 *inter alia* has recently been determined by Smales & Pate (1952), by a neutron-irradiation method. 0·024–0·047 μg atom As/l. were found, in confirmation of figures from 0·013 to 0·041 got by an indirect chemical method by Armstrong & Harvey (1950). The fraction of the arsenic present as arsenate in these water samples is not known, so that the correction to be applied to the phosphate figures is uncertain. Some analyses by Gorgy, Rakestraw & Fox (1948) of Pacific water samples of arsenic contents from 0·43 to 0·53 μg atom As/l. showed that about 10% of the arsenic was present as arsenate.

Arsenic is not included in the 'total' phosphorus figures. The validity of the 'total' phosphorus method has been tested by comparison with the perchloric acid digestion procedure of Hansen & Robinson (1953) which may reasonably be assumed to convert all organic phosphorus to phosphate. Analyses were done in sextuplicate on two samples of filtered sea water, by each of the two methods. The figures got by the autoclave method were 94 and 98% of those by the perchloric acid digestion. Dr B. H. Ketchum has stated (private communication) that he and his associates have also found satisfactory agreement between figures obtained by the autoclave method and by the sulphuric acid-peroxide digestion method of Redfield, Smith & Ketchum (1937).

Estimates of organic phosphorus may be somewhat low if they are computed from these figures by subtracting phosphate (which includes arsenate) from the corresponding 'total' phosphorus figures.

Silicate was determined by the molybdenum-blue method described previously (Armstrong, 1951). The results, which are again inherently not subject to 'salt-error', are given in microgram-atoms of silicon per litre. The factor for conversion to milligrams SiO_2 per cubic metre is 60·06.

The salinities quoted were determined at the Government Laboratory.

It is hoped that the tabulated figures will appear elsewhere in due course. The presentation here is mainly graphical, and is arranged to show the vertical distribution of temperature, phosphate, and silicate for each year. In addition, the variations of the integral mean concentrations in the water column of phosphate, 'total phosphorus' and silicate are tabulated.

OBSERVATIONS

Temperature 1950–53

Temperature diagrams are included for comparison with those for phosphate and silicate (Fig. 1).

Phosphate 1950–53 (Fig. 2)

1950. The water column was homogeneous from January until the end of April, the decrease having been rapid during the latter month. Vertical discontinuity was thereafter apparent until December, though in October and November the decrease in the upper layers was confined to the top 5 m. In July and August there was a mass of water of phosphate-content greater than 0·3 μg atom P/l., below 20 m.

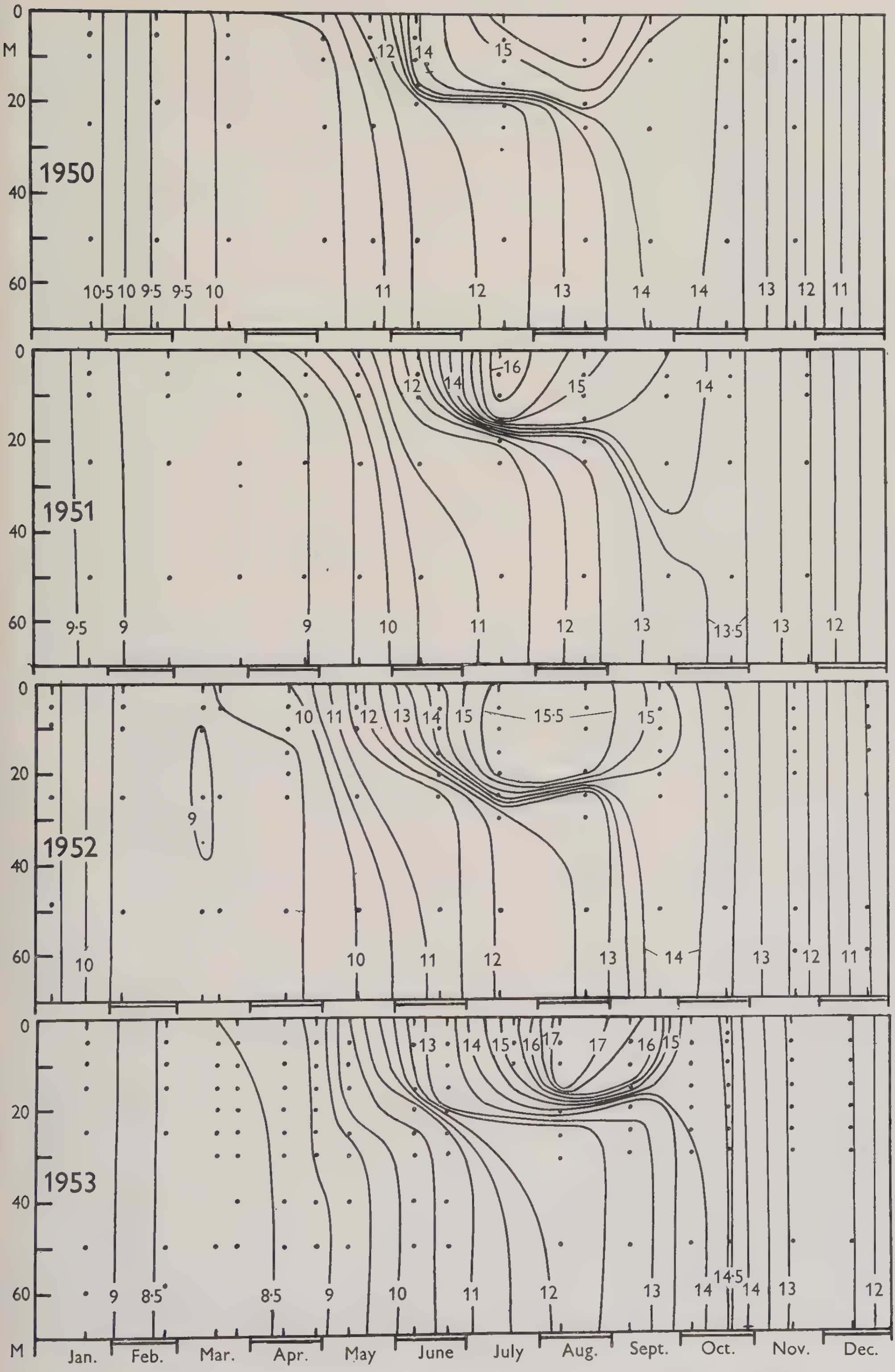

Fig. 1. Vertical temperature distribution at International Hydrographic Station E 1, 1950–53. Contour lines at 0·5° C intervals.

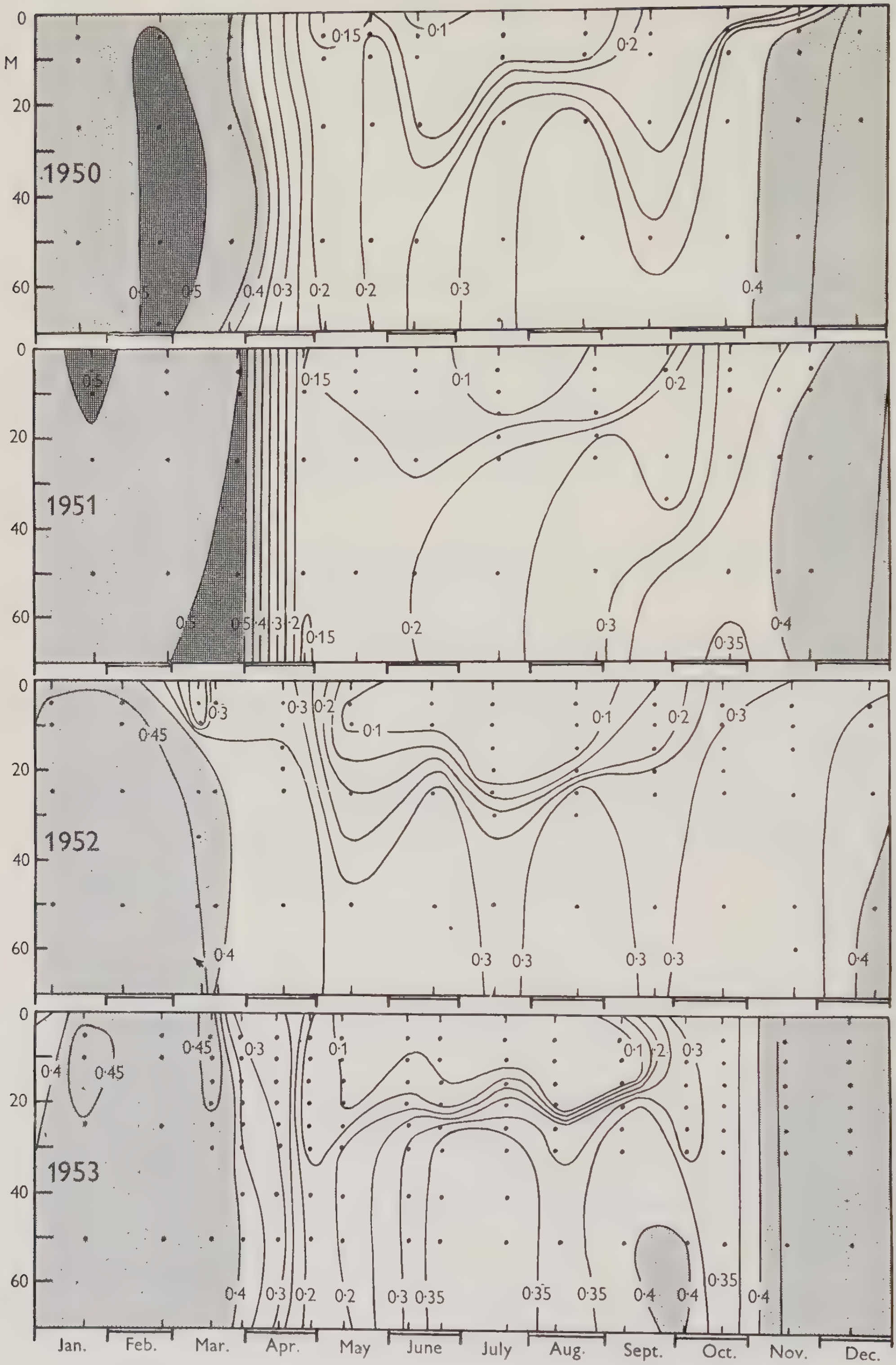

Fig. 2. Vertical distribution of phosphate, as μg atom P/l., at International Hydrographic Station E 1, 1950–53. Contour lines at 0·05 μg atom P/l. intervals.

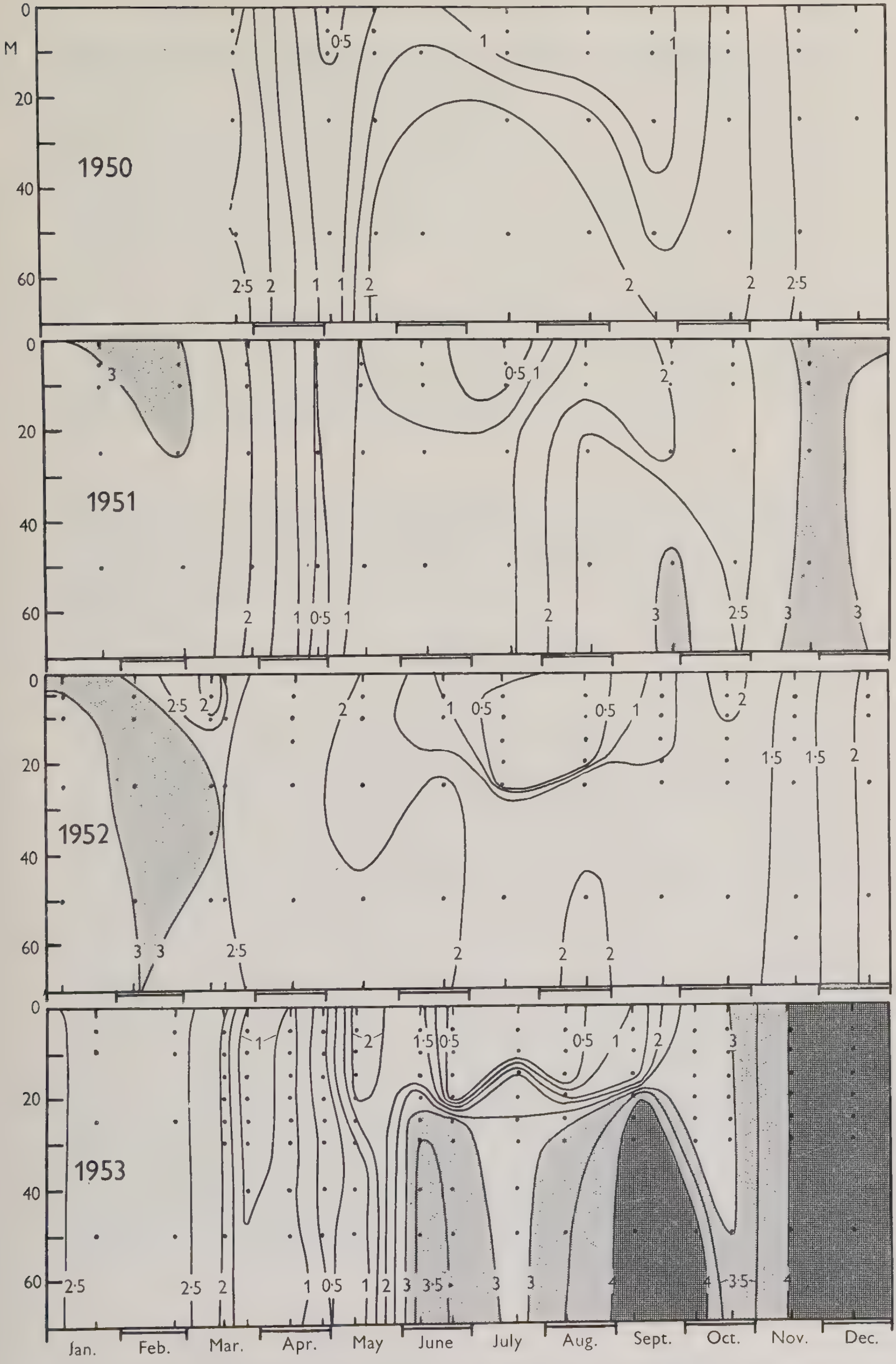

Fig. 3. Vertical distribution of silicate, as μg atom Si/l., at International Hydrographic Station E 1, 1950–53. Contour lines at 0·5 μg atom Si/l. intervals.

1951. The situation was very similar, but in the summer months there was less phosphate in the deeper water, the concentration being less than 0·3 μg atom P/l.

1952. This year differs from the other three in showing a more gradual decline in spring, with less vertical homogeneity. Depletion of the upper layers was more thorough, with a large area in the diagram showing less than 0·1 μg atom P/l., from May to August, and going to 20 m deep in July. The deeper water during the summer contained, except in July, more than 0·3 μg atom P/l.

1953. The decline in spring was more rapid than in the previous year. Stratification appeared in May, and the 0·1 μg atom P/l. contour, though not going so deep as in 1952, yet encloses a considerable area and extends from mid-May to early September. From June onward there was present below 25 m a mass of water of more than 0·3 μg atom P/l., and much of nearly 0·4.

Silicate 1950–53 (Fig. 3)

1950. Observations were started on 24 March. The water column appears to have been homogeneous during April, whilst the silicate content was decreasing rapidly, paralleling the decrease in phosphate. During the summer months stratification occurred, vertical homogeneity being re-established in October.

1951. There were slight but significant vertical differences in the column in January and February. The water then became fairly homogeneous, and silicate in the whole water column rapidly decreased to 0·5 μg atom Si/l. at the end of April. Remaining homogeneous, it rose again to over 1·0 in mid-May, after which, and until the end of October, stratification was marked.

1952. Small changes in vertical distribution were evident in January and February, whilst in March there was a marked decrease at the surface. The water became homogeneous again by mid-April. The slow decrease of this constituent parallels that of phosphate (Fig. 2). Stratification, not very marked even in May, occurred later, with a fairly heavy decrease in silicate in the upper 20 m, but the column became almost homogeneous again in September. In October there was most silicate in the *upper* layers. In November silicate was low throughout the water column, but by December the figures were up again.

1953. The diagram resembles those for 1950 and 1951, but rather different silicate concentrations occurred this year. The spring decrease took place mainly during March, which is early compared with the other years, but there was a pause in mid-April followed at the end of the month by a further fall to very low values. During all this period the vertical distribution was almost homogeneous. Early in May stratification appeared; however, it was marked by water of *high* silicate content in the upper 20 m. In June and after the upper layers were poor in silicate. On 20 July the lowest figures recorded here so far occurred in the top 10 m. At 0·5 and 5 and 10 m silicate concentrations were below the minimum perceptible with certainty by the method, i.e. were less than 0·05 μg atom Si/l.

In June, and from August onward notably high figures were observed in the deeper water.

Changes in the Integral Mean Concentrations in the Water Column

For each date on which analyses were made, the integral mean concentrations in the water column have been computed, using the formula

$$\frac{1}{2d_n}\{(a_1+a_2)(d_2-d_1)+(a_2+a_3)(d_3-d_2)+\ldots(a_{n-1}+a_n)(d_n-d_{n-1})\},$$

where $a_1, \ldots, a_n$ are observations at depths $d_1, \ldots, d_n$. The results for phosphate, total phosphorus and silicate are given in Table I.

Phosphate

The seasonal variation is well shown; sharp declines in spring occurred in April, except in 1952 when, as noted above, the fall was less abrupt. The recoveries in summer and autumn show considerable irregularity, though that of 1951 is smooth. The difference between the maximum and minimum values for each of the four years in order were 0·34, 0·35, 0·27 and 0·32 μg atom P/l. respectively.

Total Phosphorus

A seasonal variation is evident, although, as shown earlier (Armstrong & Harvey, 1950) it is less marked than that of phosphate. The differences between the maximum and minimum values for each of the four years in order were 0·27, 0·21, 0·27 and 0·15 μg atom P/l. It is not clear where this considerable quantity of phosphorus has gone. Were it used in the growth of plant or animal matter, this would amount to some 1–3 g/m^3, an amount so far unaccountable in estimates of annual production (Harvey, 1950).

Silicate

The fluctuations in this constituent were considerable, and the only general observation which may safely be made is that a decrease occurs in spring. Even so this decrease was not so steep nor rapid in 1952 as in the other years. There is a rough correspondence between changes in phosphate and silicate. It has been pointed out (Atkins, 1953) that the relative decreases in phosphate and silicate may give an idea whether diatoms or non-siliceous algae have been mainly responsible for a plankton outburst.

The most interesting of these figures were for 20 November 1952, and for 8 June and 8 September 1953 onward. Between 21 October and 20 November 1952 the mean value in the water column fell from 1·93 to 1·24 μg atom Si/l.,

TABLE I. INTEGRAL MEAN CONCENTRATIONS IN WATER COLUMN AT STATION E1

Date	Phosphate-P (μg atom P/l.)	'Total-P' (μg atom P/l.)	Silicate (μg atom Si/l.)
18. i. 50	0·46	0·57	—
22. ii. 50	0·51	0·63	—
24. iii. 50	0·46	0·54	2·53
3. v. 50	0·17	0·52	0·64
25. v. 50	0·20	0·36	1·91
12. vi. 50	0·20	—	1·60
19. vii. 50	0·28	0·42	1·88
23. viii. 50	0·32	0·48	1·68
20. ix. 50	0·28	0·46	1·07
23. x. 50	0·36	0·46	1·77
22. xi. 50	0·43	0·58	2·66
18. xii. 50	0·47	0·56	2·60
24. i. 51	0·49	0·58	2·78
26. ii. 51	0·49	0·59	2·92
28. iii. 51	0·51	0·62	1·94
25. iv. 51	0·16	0·45	0·43
17. v. 51	0·17	0·44	1·19
12. vi. 51	0·17	0·41	1·07
17. vii. 51	0·19	0·42	1·13
22. viii. 51	0·23	0·49	2·40
27. ix. 51	0·28	0·51	2·50
24. x. 51	0·33	0·46	2·39
15. xi. 51	0·40	—	—
27. xi. 51	0·40	0·53	3·19
7. i. 52	0·46	0·56	2·79
5. ii. 52	0·46	0·57	3·02
11. iii. 52	0·39	0·58	2·73
17. iii. 52	0·40	0·57	2·55
16. iv. 52	0·37	0·50	2·20
15. v. 52	0·23	0·48	2·01
19. vi. 52	0·28	0·43	1·88
15. vii. 52	0·19	0·31	1·07
20. viii. 52	0·26	0·35	1·41
22. ix. 52	0·25	0·39	1·52
21. x. 52	0·33	0·40	1·93
20. xi. 52	0·33	0·46	1·24
22. xii. 52	0·40	0·52	2·29
21. i. 53	0·45	0·56	2·69
23. ii. 53	0·43	0·56	2·72
16. iii. 53	0·44	0·53	1·96
25. iii. 53	0·36	0·53	0·86
13. iv. 53	0·30	0·51	1·13
27. iv. 53	0·15	0·41	0·26
11. v. 53	0·16	0·48	1·22
8. vi. 53	0·25	0·49	3·02
22. vi. 53	0·29	0·51	2·31
20. vii. 53	0·31	0·52	2·08
10. viii. 53	0·23	0·41	2·36
8. ix. 53	0·32	0·42	3·41
5. x. 53	0·34	0·49	3·50
20. x. 53	0·32	0·42	3·06
17. ix. 53	0·47	0·52	4·05
14. xii. 53	0·47	0·54	3·90

with no corresponding change in phosphate. This fall in silicate cannot readily be ascribed to a late autumn outburst of diatoms because at the same time the total amount of chlorophyll in the water column decreased (Atkins, Jenkins & Warren, 1954).

On 8 June and on and after 8 September 1953, silicate figures were notably higher than at the beginning of the year. These high integral mean concentrations do not of course include any silicate sequestered by diatoms, yet silicate in solution has increased in the column as a whole.

The salinity determinations give little support to the idea that these results are caused by changes in the body of water sampled at E1. It is, however, possible that enrichment (not the impoverishment noted in November 1952) could be caused by solution of siliceous matter suspended in the water. Proximate analyses of this matter have shown (Armstrong & Atkins, 1950) that at the surface it may account for 1·3–14·7 μg atom Si/l., thus solution of a fraction only of this silica would account for the increases found. It has indeed been shown (Armstrong, 1950; Atkins, unpublished) that the soluble silicate content of sea water increases on standing (in non-siliceous bottles).

During 1953, such a process of solution would need to have been rapid to cause the changes seen between 11 May and 8 June and between 10 August and 8 September. It is odd too that this is the only year in which the effect is seen.

Changes in Salinity

Small changes in salinity occur frequently in the records for these four years, and do not necessarily show that different water masses have been sampled at different times. Since the water at E1 is only some 70 m deep, measurable changes in salinity throughout the water column may be caused by heavy rainfall.

At the end of 1952 and in the latter half of 1953, the integral mean salinities were as shown in Table II.

TABLE II. SALINITIES AT E1 IN 1952 AND 1953

Date	Integral mean salinity (‰)	Number of observations for computation
21. x. 52	$35\cdot22_{3}$	8
20. xi. 52	$35\cdot14_{8}$	8
22. xii. 52	$35\cdot17_{6}$	7
11. v. 53	$35\cdot15_{9}$	10
8. vi. 53	$35\cdot16_{5}$	10
22. vi. 53	$35\cdot14_{2}$	10
20. vii. 53	$35\cdot12_{8}$	10
10. viii. 53	$35\cdot12_{1}$	9
8. ix. 53	$35\cdot16_{1}$	9
5. x. 53	$35\cdot20_{6}$	9
20. x. 53	$35\cdot21_{0}$	9
17. xi. 53	$35\cdot21_{0}$	9
14. xii. 53	$35\cdot26_{1}$	9

It is probably justifiable to assume that the water mass sampled in November 1952 was different from that sampled in October. Evidence from salinity measurements in 1953 is inconclusive, but the increase in salinity from October onward was accompanied by a change in silicate content to values greater than at the previous winter maximum.

Attention has been given to the possibility of change in the water body at E 1, because changes of water masses, possibly with different stocks of nutrient salts, may invalidate arguments based on the apparent utilization of these nutrients.

On 11 March 1952 (the year which was unusual in showing a slower decrease in phosphate and silicate in spring) there were plainly two water masses, of different densities, one above the other at the Station. This is shown by the figures of Table III.

TABLE III. SALINITY AND DENSITY AT E 1 ON 11 MARCH 1952

Depth (m)	Temperature (° C)	Salinity (‰)	Density *in situ* (σ_T)
0·0	9·1	35·13	27·22
5	9·04	35·09	27·20
10	9·00	35·09	27·20
25	8·95	35·21	27·20
35	8·98	35·27	27·35
50	9·11	35·26	27·33
68	9·11	35·28	27·34

TABLE IV. ALTERNATE SILICATE OBSERVATIONS, E 1, 1953

Date	μg atom Si/l.	Date	μg atom Si/l.
21. i. 53	2·69	21. i. 53	2·69
23. ii. 53	2·72	23. ii. 53	2·72
25. iii. 53	0·86	16. iii. 53	1·96
27. iv. 53	0·26	13. iv. 53	1·13
11. v. 53	1·22	11. v. 53	1·22
22. vi. 53	2·31	8. vi. 53	3·02
20. vii. 53	2·08	20. vii. 53	2·08
10. viii. 53	2·36	10. viii. 53	2·36
8. ix. 53	3·41	8. ix. 53	3·41
5. x. 53	3·50	20. x. 53	3·06
17. xi. 53	4·05	17. xi. 53	4·05
14. xii. 53	3·90	14. xii. 53	3·90

It is instructive also to consider an aspect of the silicate figures for 1953. Analyses were done more frequently than usual since samples were taken when the ships were at the Station for other purposes. It is possible to choose alternate dates at roughly monthly intervals, to make two series of observations (Table IV). Each series gives twelve observations in 12 months, but the conclusions drawn from the two series might be very different. Phosphate and 'total phosphorus' do not show such marked differences.

The desirability of making frequent and complete observations is obvious, even in so simple a task as logging the changes at a single station.

SUMMARY

Samples taken at the International Hydrographical Station E 1 during the last four years have been analysed by recently developed methods.

The results for phosphate, 'total phosphorus', and silicate are presented graphically, showing the seasonal changes in vertical structure and integral mean concentration in the water column.

The figures are discussed, particular note being made of unusual silicate values in 1952 and 1953.

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J. Mar. biol. Ass. U.K. (1954) 33, 393-401
Printed in Great Britain

HATCHING IN *CALANUS FINMARCHICUS* AND SOME OTHER COPEPODS

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(Plate I and Text-figs. 1–5)

During observations on the development of the eggs of *Calanus finmarchicus* the process of hatching was watched on many occasions. Since it differs from that described by Ziegelmayer (1926) for cyclopids it seemed worth while examining the process in other groups of copepods as well.

Hatching can be followed very easily in *Calanus* (Pl. I). For some time before, the form of the nauplius can be clearly seen with its limbs folded close to the body (A); in many a red pigment spot develops at this time in the otherwise transparent egg. Shortly before hatching the limbs begin to twitch occasionally and a space is just visible round the body of the nauplius. At this stage the inner and the outer egg membranes cannot be distinguished, but when hatching begins, the outer membrane must crack for the delicate inner membrane bulges out (B). The bulge enlarges rapidly (C), and the nauplius extends into the bulge, sometimes leaving a clear space between itself and the membranes (D). Usually it is either the head or the tail end which pushes into the bulge, but occasionally it is a limb. Probably it is abnormal for a limb to extrude first since such eggs do not always hatch successfully. As the inner membrane expands, the outer slips back and the inner, enclosing the nauplius, pops out (E). This emergence often occurs suddenly, but sometimes it is gradual (as in E), when the outer membrane crumples up on the inner, regaining its original shape only when the inner has slipped out and the tension is released (F). The inner membrane now forms a perfect sphere of much greater diameter than the original egg.

During the first part of the hatching process the nauplius continues to twitch at irregular intervals. After it has emerged in its inner membrane, it has a considerable space round it, and it then begins to stretch out its limbs and become more active (G). Quite suddenly it tears the membrane and swims away (H). The diaphanous remains of the inner membrane are hardly visible. The whole process takes about 10–15 min at room temperature (15° C), but the time spent in each stage is variable. Sometimes longer is spent in hatching from the outer membrane and sometimes longer from the inner.

Since the diameter of the egg is $145\,\mu$ its volume is about $0{\cdot}0016$ mm.^{3}. Two eggs, in which the inner membrane was measured immediately after slipping out, had diameters of 191 and 209 μ, the volumes being now more than double, 0·0037 and 0·0048 mm.^{3} respectively. The diameter increased and was measured again just before the nauplius escaped, when it was for both eggs 244 μ, giving a volume of 0·0076 mm.^{3} or nearly five times the original. The surface area had therefore increased 2·8 times. One nauplius was measured a few minutes after hatching and was 174 μ long, i.e. larger than the original diameter of the egg.

Osmotic hatching has been suggested in a number of Crustacea (see Yonge, 1937), and we therefore examined the possibility in *Calanus* eggs. Twenty-nine eggs near hatching were used for the experiment; fourteen were kept in normal sea water as controls and fifteen in sea water to which a small quantity of fresh water was added. In the controls three bulged and hatched successfully; in the fifteen which were treated experimentally, eleven bulged although only seven of these hatched successfully. It is obvious, therefore, that osmosis may be an important factor in hatching. If fresh water is added some time before the nauplii are ready to hatch, the egg ruptures, extruding a mass of tissue, but the inner membrane does not appear at all.

C. helgolandicus eggs hatch in exactly the same way. In another very similar form of *Calanus*, found at Tromsø (Marshall, Orr & Rees, 1953), eggs are laid which produce, shortly before the first division (possibly on fertilization), an extra, thin, irregular, membrane separated by a varying space from the original egg. Hatching of these takes place in the same way as in other *Calanus*.

The eggs of *Metridia longa* and *Acartia clausi* also hatch in this way. In *Acartia* the outer membrane seems to be relatively more rigid than in *Calanus*. It does not crumple, but splits and slips off by gaping wider. The inner membrane also seems relatively thicker since it is quite clearly visible after the nauplius has left it. Shortly before hatching a rosy tinge becomes visible over about half the egg, and in the hatched nauplius the tips of the antennules are pink. The eggs measure 77–78 μ before hatching begins, and in one the inner membrane just before the nauplius escaped had swelled to 129 μ thus increasing the volume four and a half times.

EXPLANATION OF PLATE I

A. Egg of *Calanus finmarchicus* shortly before hatching showing the nauplius form.

B. The inner membrane begins to bulge. The outer membrane has a diatom chain attached which can be seen throughout the rest of the series.

C. The bulge of the inner membrane is growing rapidly.

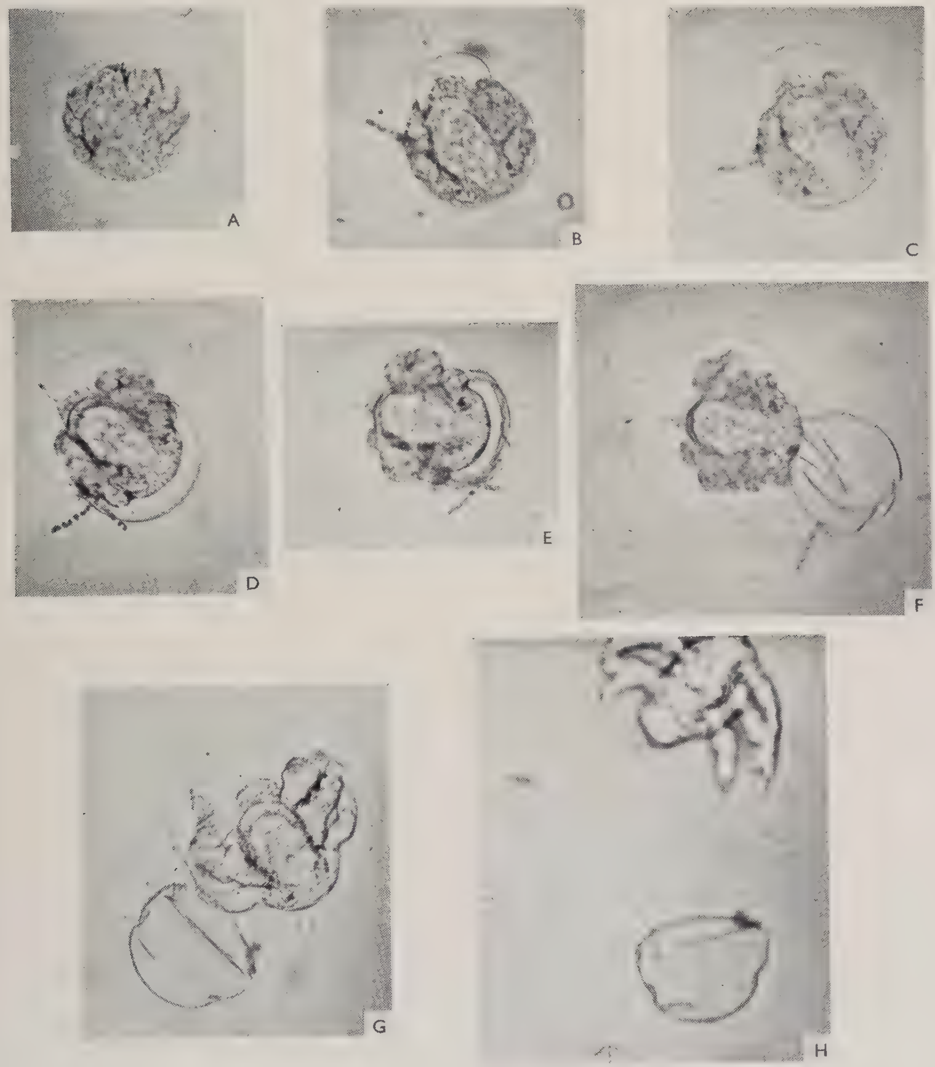
D. The nauplius inside the inner membrane is emerging from the outer.

E. The outer membrane has crumpled up and the diameter of the inner membrane much exceeds that of the original egg.

F. The outer membrane has sprung off and has partly regained its spherical form. There is a large space between the nauplius and the inner membrane.

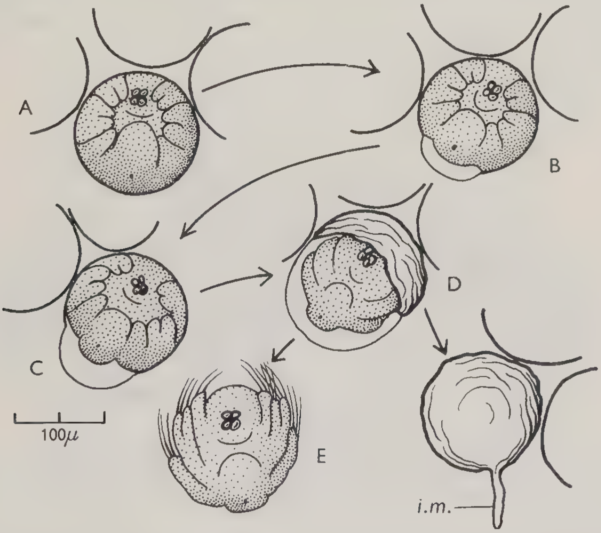
G. The nauplius stretches its limbs.

H. The nauplius is swimming away and the remains of the inner membrane can be faintly seen, indicated by the arrows.


A
B
C
D
E
F
G
H

All these copepods liberate their eggs directly into the sea. Others, however, carry them in sacs attached to the genital segment, and several of these species were observed to see how hatching takes place.

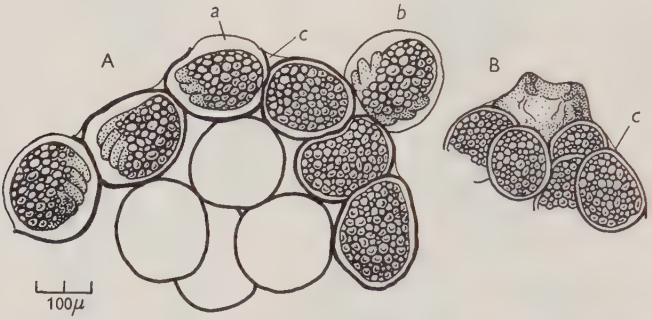
The female of *Pseudocalanus minutus* carries a mass of eggs varying in number according to the season from about 10 to 28 (Marshall, 1949). As the eggs develop they get darker in colour, and in some a red pigment spot is seen. The process of hatching is much the same as that already described; the outer membrane cracks, the inner membrane bulges out and eventually the nauplius comes out (Text-fig. 1). Usually, however, the inner membrane does not come completely free from the outer before the nauplius struggles and breaks it.



Text-fig. 1. Hatching in *Pseudocalanus minutus*. A, egg shortly before hatching; B, inner membrane beginning to bulge out; C, a later stage; D, outer egg-membrane crumples up and nauplius emerges in inner membrane; E, nauplius escapes and inner egg membrane (*i.m.*) is left attached to outer.

The torn inner membrane usually remains attached to the outer. Once the inner membrane enclosing the nauplius came right out and floated away. It swelled until there was a space round the nauplius but this egg failed to hatch. The eggs before hatching measured between 120 and 140 μ and the inner membrane increased up to 160–170 μ in diameter, an increase of a little more than twice the volume. The swelling is therefore not so great as in *Calanus*. Although the egg-mass often looks as if it were contained in an actual sac, when the nauplii have hatched out it seems to be composed only of individual outer membranes stuck together.

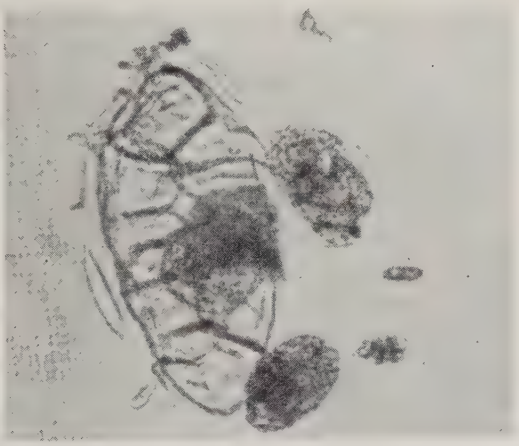
Euchaeta norvegica is a large copepod living in deep water and it does not survive well under laboratory conditions. The eggs when first laid are deep blue in colour, and according to Nicholls (1934) a normal egg-sac contains about 50 eggs. Development is slow, and the interval between laying and hatching is at least 8 days compared with the 24 h normal for *Calanus*. It was therefore not unexpected that the process of hatching should be prolonged. As development goes on the eggs change in colour from dark blue to reddish brown, and the body of the nauplius is seen to be full of fat globules. As usual the outer membrane eventually cracks and the inner membrane bulges out (Text-fig. 2A). Nauplii may remain in this condition for many hours. On one occasion a nauplius had broken its inner membrane and was partly hanging out, twitching actively, when a sudden jerk on the part of the mother threw it right out. For a minute the remains of the inner membrane were to be seen clinging round the hind end and then the nauplius left them behind and swam actively away. Another nauplius enclosed in its inner membrane was thrown out at the same time.



Text-fig. 2. Hatching in *Euchaeta norvegica*. A, nauplius (*a*) bulging out in inner membrane; and (*b*) in inner membrane almost free from egg-sac; B, collar of secretion which attaches egg-sac to genital segment. *c*, cuticular secretion.

One female in which the eggs were hatching was kept overnight. In the morning there were about twenty-two nauplii in the surrounding water, most free and active but several enclosed in their inner membranes. They remained like this for some time and actual hatching was not seen. In detaching an egg-sac from a dead female a number of nauplii, some in the inner membrane and some free, were shaken out, and it seems likely that the movements of the mother play an important part in helping the nauplii to hatch. The egg-sac, which is flattened in form, is attached to the genital segment by a projecting collar of secretion (Text-fig. 2B).

In the cyclopids the eggs are carried in paired sacs attached to the genital segment. In *Oithona similis* the egg is oval, the diameter varies from 70 to 95 μ, and each sac contains from five to eighteen eggs according to the season. The process of hatching is not so easily followed as in a larger egg, and there are several differences from *Calanus*. When the outer membrane cracks the inner membrane cannot be seen bulging out. It envelops the nauplius much more closely than in *Calanus*, and the only sign of the outer membrane rupturing is a sudden stretch on the part of the nauplius up to 105 μ or more. After a short time the nauplius slides forwards and pushes well out of the outer membrane, sometimes twisting round as it does so. A female has been seen swimming about with the nauplii hatching and hanging out in all directions round the egg-sac. The inner membrane cannot always be made out at this stage, but is seen most clearly when the nauplius is viewed from the side. After quite a long pause, during which the nauplius twitches frequently, it stretches out its limbs (which up till now have been close to the body) and struggles to get its spines loose. When it finally breaks free and swims off, the inner membrane is usually left attached to the outer.



Text-fig. 3. Photograph of the egg-sac of *Cyclops agilis*. Most eggs have hatched, leaving the inner membranes hanging on to the outer. A nauplius still in its inner membrane is seen on the right.

In the freshwater copepod *Cyclops agilis* the eggs measure 90–110 μ in diameter. The nauplius was not seen to twitch before hatching. When the outer membrane cracks the inner membrane bulges out for an instant and then almost immediately slips right out, although still remaining attached to the outer. It fits the enclosed nauplius fairly closely; it is not spherical and can be seen most clearly when the nauplius is viewed from the side, for although touching the head and tail it is well separated dorsally and ventrally (Text-fig. 3). Very little swelling takes place, the maximum diameter being only 120 μ and the volume is thus only doubled. After a few minutes, during which it twitches occasionally, the nauplius gives a violent movement, breaks out and swims away, almost always leaving the inner membrane attached to the outer. The inner membranes seem to be relatively thicker than in *Calanus* and, after all the nauplii have hatched, they can be seen hanging out all round the egg-sac.

In *Cyclops viridis*, another freshwater copepod with eggs about 130 μ in diameter, hatching takes place more slowly. The inner membrane bulges out (Text-fig. 4A) and, as it swells, the outer membrane can be seen crumpling up (Text-fig. 4B), as it sometimes does in *Calanus* (Pl. IE), springing back to its original shape when the nauplius pops out in its inner membrane. This stretches to contain nearly four times the original volume and, as it does so, the nauplius increases in size so as to fill it completely (Text-fig. 4C). When the nauplius breaks out and swims away, it usually leaves the inner membrane attached to the outer (Text-fig. 4D), but sometimes leaves it free in the water. All the eggs in one egg-sac hatch at practically the same time, and the violent movements of the hatching nauplii jerk the detached egg-sac about.

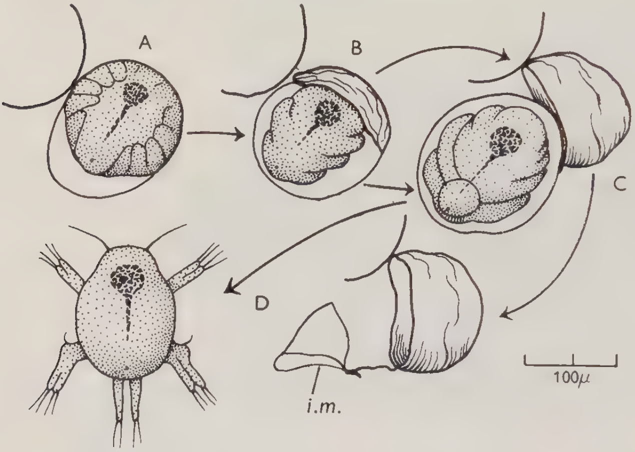


Fig. 4.

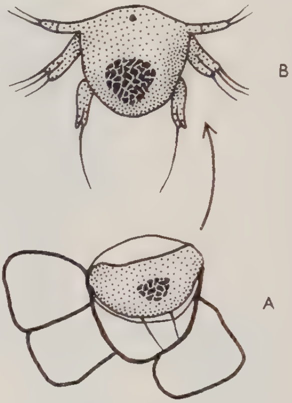


Fig. 5.

Text-fig. 4. Hatching in *Cyclops viridis*. A–C, stages in hatching; D, nauplius and egg-shell with inner membrane (*i.m.*) still attached.

Text-fig. 5. Hatching in *Tigriopus fulvus*. A, the nauplius bulging out in its inner membrane; B, nauplius showing the long mandibular spines.

A harpacticid *Tigriopus fulvus* was also observed. There is a single egg-sac and, as in *Cyclops viridis*, the eggs all reach the hatching stage at about the same time. The nauplii twitch occasionally for about an hour before emerging. The outer membranes crack, the inner membranes bulge out, and in a short time the whole sac is covered with nauplii hanging out in their inner membranes (Text-fig. 5A). A curious feature is that the long mandibular spines seem to extend beyond the inner membrane and remain attached to the inside of the outer membrane. The inner membrane disappears suddenly, but whether it bursts or whether the nauplius increases to fill it completely is uncertain. The nauplius is still firmly attached by the mandibular spines and violent struggles are necessary to detach them. After all the eggs have hatched a few inner membranes are seen hanging on the outer membrane, but they are not so many nor so distinct as in *Cyclops*.

In the semi-parasitic copepod *Caligus rapax*, the egg-sacs are each formed of a long row of single eggs one behind the other, and hatching takes place in the same way. The eggs hatch successively from behind forward and each bulges out in the inner membrane for a few minutes before breaking out and swimming off. According to Mr B. T. Hepper of the Burnham-on-Crouch Laboratory (personal communication) the eggs of *Mytilicola intestinalis*, a copepod parasitic in mussels, hatch in a rather similar way.

Discussion

It is clear that in all these copepods, belonging to several different groups, hatching takes place on the same general plan. As development goes on there is often a colour change in the nauplius (*Calanus*, *Pseudocalanus*, *Euchaeta*, *Acartia*), and this is sometimes the first sign of hatching. The rigid outer egg-membrane is first burst by the pressure inside the inner membrane. This inner membrane, enclosing the nauplius, then emerges from the outer membrane, but it may do so gradually as in *Calanus* or very quickly as in *Cyclops agilis*. The increase in volume which takes place inside the inner membrane varies with the species. In *Calanus* the volume increases about fivefold before the nauplius hatches, but in eggs which are carried in egg-sacs the increase is usually much smaller and sometimes may be little more than is necessary to crack the outer shell. In some, e.g. *Calanus*, there is a large clear space round the nauplius after it has slipped out, while in others such as *Oithona* the nauplius fills the inner membrane completely; the other cyclopids are intermediate in that they seem to fill it when viewed dorsally or ventrally but not when viewed laterally. In both types the nauplius stretches or imbibes water before tearing the inner membrane, but the first does so at a later stage than the second.

In those copepods which lay eggs freely in the sea (*Calanus*, *Metridia*, *Acartia*) the inner membrane separates completely from the outer, but in those hatching from egg-sacs it more commonly remains attached. This may help the nauplius to avoid entanglement. It is not clear how the inner membrane stays attached, for it often (see Text-figs. 3 and 4) seems to come right out of the outer. *Euchaeta norvegica* is a copepod which shows both methods. Some of the nauplii in their inner membranes come out quite clear of the egg-sac before hatching, while others leave their inner membranes attached. The movements of the female in this and other copepods may have some effect in helping nauplii to escape from the egg-sac.

A puzzling feature is that in *Oithona* and *Tigriopus* the spines seem to extend through the inner membrane. This follows the outline of the nauplius body when the latter is hanging out from the outer shell, but the spines project beyond it and seem to be attached to the inside of the outer membrane (see Text-fig. 5A). The nauplii have a considerable struggle to release the spines before they escape.

Ziegelmayer's (1926) observations on the hatching of seventeen species of cyclopids differ considerably from ours. According to him it is the outer egg-membrane which swells. This process may take 14 h or more. A pressure develops in the space thus formed between the outer and the inner membranes. The outer membrane eventually bursts and the inner, in which the nauplius is tightly enclosed, emerges. After a short time the nauplius moves its antennae, tears the inner membrane, and escapes; it then remains still for 10–20 sec, begins to move its limbs, and finally swims off.

According to our observations, however, it is the inner membrane and not the outer which expands. It is difficult to decide whether Ziegelmayer was unable to see the bulging out of the inner membrane or whether the specimens he examined behaved in a different way. The eggs in his two photographs, taken after 3 and 10 h swelling, are, when we allow for the different magnifications, very much the same in diameter, and the second resembles a nauplius released in its inner membrane.

That hatching may be an osmotic process is suggested by the few experiments on *Calanus* eggs described above, and this has been found also in cyclopid copepods by Ziegelmayer (1926), and in several other Crustacea—e.g. in *Hemimysis* by Manton (1928), and in *Daphnia* and *Simocephalus* by Przylecki and Ramult (Needham, 1931).

A possible explanation of the mechanism of hatching might be that a sudden increase of excretion by the embryo leads to an increased content of salts and the imbibition of water. It is noteworthy that in nearly all species twitching of the nauplius begins shortly before hatching. It is difficult, however, to understand how osmotic control can be effective in *Tigriopus*, which lives in shore pools and is therefore subject to considerable variations in salinity.

In the eggs of decapods (Yonge, 1937, 1946) there are two membranes, a cuticular, secreted by glands on the pleopods of the female, and a chitinous, secreted by the oviduct. According to Herrick (1895), the cuticular membrane splits at hatching and completely separates from the underlying membrane except at one point. This underlying membrane (presumably the chitinous one of Yonge) is now greatly distended and from it the young lobster hatches.

In most copepods that bear egg-sacs only two membranes are visible. *Euchaeta norvegica* is the only species we have used that is large enough to give clear results with chemical tests. In it the mass of secretion joining the egg-sac to the genital opening, together with the material connecting the eggs (Text-fig. 2B, *c*), has been shown to be cuticular in nature. This cuticular secretion presumably also surrounds each egg. In *Caligus* (Wilson, 1905) cement glands are present which secrete the egg-sac and also a covering round each egg.

It seems most probable that in the copepods the two membranes we have described as outer and inner correspond to the cuticular and chitinous membranes in the lobster, the chitinous one being extensible.

An alternative explanation might be, however, that in copepods the extensible inner membrane is secreted by the developing embryo, and that the chitinous and cuticular membranes are so closely adherent that they are indistinguishable except possibly in *Euchaeta*.

We are grateful to Prof. C. M. Yonge, F.R.S., for his interest in the problems raised and especially for his help in the chemical tests. We should also like to thank Dr J. P. Harding of the British Museum for identifying the species of *Cyclops*, *Tigriopus* and *Caligus*, and Dr R. B. Pike for preparing the drawings for reproduction.

SUMMARY

The process of hatching is described for *Calanus finmarchicus* and other copepods. In *Calanus* the outer egg membrane breaks and the nauplius emerges enclosed in a very delicate inner membrane which swells to contain a volume as much as five times that of the original egg. The nauplius escapes by tearing this membrane. Other copepods which lay their eggs freely in the sea hatch in the same way, but in copepods that carry egg-sacs the swelling in the inner membrane is less, and when the nauplius emerges the inner membrane is usually left attached to the outer.

The mechanism of the hatching process and the nature and origin of the membranes are briefly discussed.

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J. Mar. biol. Ass. U.K. (1954) 33, 403–407
Printed in Great Britain

THE DEVELOPMENT OF THE ASCIDIAN *PYURA MICROCOSMUS* (SAVIGNY)

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(Text-figs. 1 and 2)

Several recent papers have dealt with the development of members of the ascidian family Pyuridae (Hirai, 1941; Berrill, 1948; Millar, 1951; Sebastian, 1953), but the total number of species with known larvae is still only five, in spite of the large size of the family.

The present paper deals with the embryonic development, the larva, and the post-larval development of *Pyura microcosmus* (Savigny).

Material and Method

On 30 July 1953 specimens of *Pyura microcosmus* were collected from stones about the level of zero tides, on the north shore of Loch Creran, Argyll, on the Scottish west coast. The animals were placed in a large vacuum jar of sea water and taken on the following day to the Marine Station, Millport. Artificial fertilizations were made by mixing eggs from the oviduct of one individual with sperm from the sperm duct of another. The development obtained from three separate pairs of animals was closely similar. Shortly after fertilization the water was changed to remove excess sperm, and during the course of development two more changes of water were made. The temperature of the water varied from 18·0 to 20·0° C.

Embryonic Development

The ripe ovum is spherical and about 200 μ in diameter, with pink-grey cytoplasm and a clear germinal vesicle. The ovum is surrounded by a layer of irregular outer-follicle cells, each containing a few grains of rose-purple pigment, and by scattered inner-follicle cells. The two layers of follicle cells are separated by the thin chorion which in newly extracted eggs has a diameter of about 235 μ. After a short time the perivitelline space enlarges and the diameter of the chorion consequently increases.

After fertilization the germinal vesicle disappears and a grey-brown cap is formed on one side of the ovum, with a pale centre and a surrounding ring of clear cytoplasm. Before the first cleavage the grey-brown cap becomes crescentic and it is shared between the first two cells during cleavage (Fig. 1 A). This grey-brown cytoplasm corresponds to the yellow crescent of *Styela* *partita* originally described by Conklin (1905), and the variously coloured crescent of other ascidians (Hirai, 1941; Berrill, 1948; Millar, 1951; Sebastian, 1953). Subsequent cleavage is essentially the same as that of styelid and pyurid ascidians already studied. The larva, still within the chorion, is fully developed and showing muscular twitchings within 13 hours of the mixing of eggs and sperm. Hatching follows in the next few hours.

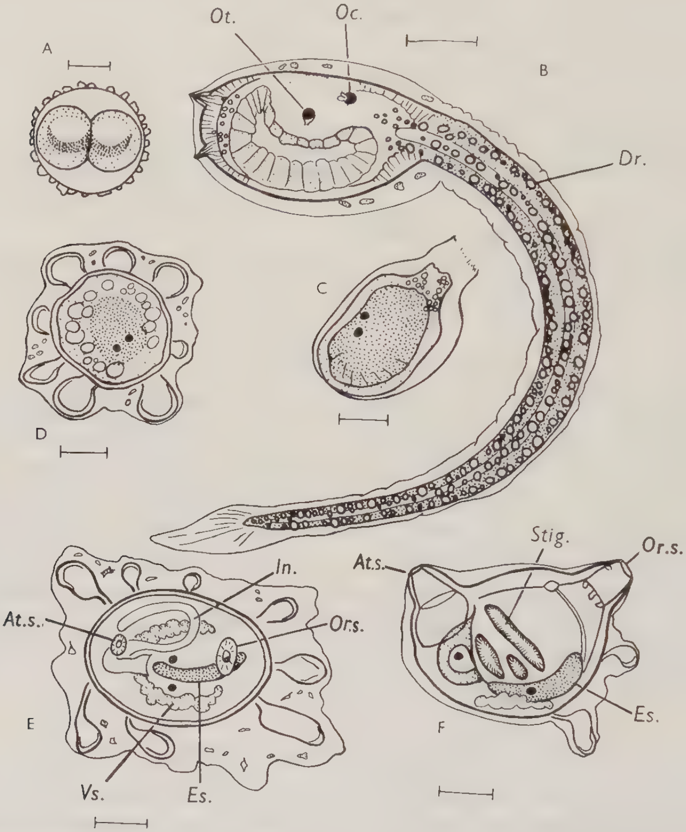

Fig. 1. Development of *Pyura microcosmus* (Savigny). A, two-cell stage, showing dark crescent; B, larva; C, larva during metamorphosis; D, dorsal view of metamorphosing larva, with ring of ampullae; E, dorsal view of young ascidian after differentiation of gut; F, lateral view of young ascidian. The scales represent 100 μ. *At.s.*, atrial siphon; *Dr.*, refringent droplets; *Es.*, endostyle; *In.*, intestine; *Oc.*, ocellus; *Ot.*, otolith; *Or.s.*, oral siphon; *Stig.*, anterior of the first three stigmata of the branchial sac; *Vs.*, crescent of vesicular cells.

LARVA

The larva (Fig. 1 B) is divided into a trunk about 285 μ long, and a tail about 1000 μ long. A layer of clear test covers the surface of the larva and is developed into a vertical fin along the dorsal and ventral sides of the trunk and tail. A leaf-like expansion of the fin projects beyond the end of the tail. At the anterior end of the trunk are two dorsal and one ventral adhesive papillae. The rudiment of the pharynx lies in the ventral half of the trunk. Near the dorsal surface of the trunk is the sensory vesicle, containing on its posterior wall the ocellus and on its ventral wall the otolith. The ocellus (Fig. 1 B, *Oc.*) has a black cup-shaped mass of pigment 14μ in diameter, behind which are the retinal cells. Clear lens cells project from the cavity of the pigment cup. The otolith (Fig. 1 B, *Ot.*) is a spherical black body $13{\cdot}2\mu$ in diameter contained in a single pear-shaped cell which projects upwards from the floor of the sensory vesicle.

Many small refringent droplets are present in the trunk, mainly in the posterior regions; it is not certain which cells contain these droplets.

In the tail are the usual structures of the ascidian larva: a central notochord, dorsal neural strand, and three bands of striated muscle on each side. A longitudinal row of refringent droplets (Fig. 1 B, *Dr.*) is associated with each of the muscle bands of the tail.

METAMORPHOSIS AND POST-LARVAL DEVELOPMENT

Within 24 hours of hatching some larvae have started to metamorphose, although others still show no sign of change. Attachment of larvae to a substratum is not necessary for metamorphosis, which occurs in attached and unattached larvae. The first change is reduction of the tissues of the tail (Fig. 1 C), but not the covering test which retains its original form and persists long after metamorphosis is complete. While the tail is shrinking the trunk produces a ring of anterior ampullae which are applied to the substratum (Fig. 1 D). Typically these ampullae number eight, but some variation is found. They grow outwards as long finger-like structures, and simultaneously a disk of expanding test spreads over the substratum. Meanwhile other changes occur in the body, and a central dark mass of cells appears, surrounded by large vesicular cells. The larval sensory pigment is still present within the body of the developing ascidian. The dark central mass now elongates and differentiates to form the rudiment of the pharynx and gut, and the surrounding vesicular cells become arranged in a crescent on each side of the pharynx. At an early stage in the formation of the pharynx the endostyle is recognizable, and shortly afterwards the oral and atrial siphons are formed (Fig. 1 E). The atrial siphon is, from the first, a single structure, and is not derived from the fusion of paired peribranchial openings. With the further differentiation of the gut to form the oesophagus and stomach, and the intestinal loop on the left of the pharynx, and with the perforation of one pair of protostigmata, the essential organization of the adult is accomplished. The original protostigmata divide to form two, then three, pairs of openings (Fig. 1 F). Up to about this stage the larval pigment of the ocellus and otolith remain unchanged within the body of the young ascidian, but now they are transported out of the body and deposited either on the outer surface of the test, or, more usually, quite outside the body and often at some distance from it (Fig. 2). This remarkable movement of the pigment spots, which was observed in each of the young ascidians examined, is presumably accomplished by phagocytes which, having ingested the pigment, migrate out of the body.

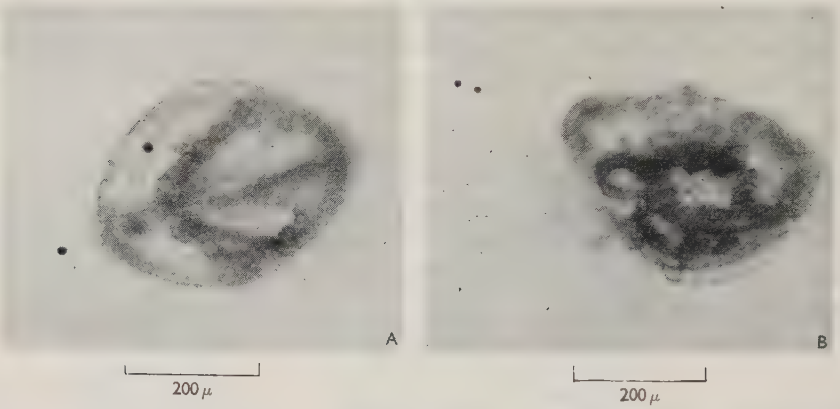

Fig. 2. Development of *Pyura microcosmus* (Savigny). A, dorsal view of young ascidian showing outward migration of one of the larval pigment spots. B, young ascidian after migration of both pigment spots out of the body.

The heart by now is differentiated, and starts to beat. It already shows the reversal characteristic of ascidians, although at this early stage it changes direction at irregular intervals.

Shortly after, a circle of spines develops round each of the siphons, this being the first sign of the elaboration of the test typical of the Pyuridae.

TABLE I. TIME-TABLE OF DEVELOPMENT

Stage	Time
Eggs and sperm mixed	11.00 a.m., 3. viii. 53.
First cleavage	12.20 p.m.
Second cleavage	12.45 p.m.
Gastrula	4.45 p.m.
Closure of blastopore	5.45 p.m.
Trunk and tail differentiated	8.00 p.m.
Larva complete, and active within chorion	11.45 p.m.
Larva hatched and active	9·00 a.m., 4. viii. 53.
Metamorphosis: tail reduced and ampullae forming	5. viii. 53.
Tail resorbed, ampullae enlarging	6. viii. 53.
Endostyle differentiated	10. viii. 53.
Siphons and gut differentiated	11. viii. 53.
One pair of stigmata present and active	12. viii. 53.
Three pairs of stigmata; removal of larval sensory pigment outside body; heart beating	14. viii. 53.
Elaboration of test spines on siphons	17. viii. 53.

Table I shows the times at which important stages were reached in development.

The general course and timing of development agree more closely with those of *Pyura squamulosa* (Alder) than with any of the other pyurid ascidians whose development has been studied. The egg of *P. microcosmus*, however, is larger than that of *P. squamulosa*, and the larva also correspondingly larger.

SUMMARY

The embryonic development, larva, and post-larval stages are described in the ascidian *Pyura microcosmus* (Savigny). The development is similar to that of *P. squamulosa* (Alder), but the egg and larva are larger. In the young ascidian the persisting pigment of the larval ocellus and otolith are transported out of the body, presumably by the action of phagocytes.

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J. Mar. biol. Ass. U.K. (1954) 33, 409–448
Printed in Great Britain

EXPERIMENTAL OBSERVATIONS ON THE VERTICAL MIGRATIONS OF PLANKTON ANIMALS

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(Plates I and II and Text-figs. 1–29)

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Much information concerning the vertical migration of plankton animals has been gained by using nets at different levels in the sea and at different times of the day and night, and also by experimenting with animals in apparatus of various kinds both in the laboratory and in the sea itself. Such observations and experiments have been summarized in the extensive reviews by Russell (1927) and Cushing (1951) who give full lists of references.

In all this earlier work a limited knowledge of the migrations was obtained by inference. It had not been possible actually to see plankton animals making their extensive upward or downward journeys, so that little was known of how they accomplished them, whether for example by swimming directly and continuously upward or by making more random movements with a general upward tendency. There were no direct data regarding the speed or duration of their climbing and it remained an open question whether they descended passively by sinking or actively by swimming down. Such observations would normally require a vertical column of water of great length. The present paper describes the results of experiments and observations made with a new type of apparatus designed to overcome this difficulty and to give answers to some of these questions.

Before the war, with the help of a grant from the Leverhulme Trustees, Hardy & Paton (1947) began studies on vertical migration by using glass cylinders in the sea. This work was interrupted by the war and Neil Paton was killed in action. The Leverhulme Trustees kindly allowed the remaining portion of their grant to be used in the present investigation. The equipment about to be described is related in its origin to the long tube apparatus of the former investigation; it is in fact such a long tube bent in a circle to form a continuous channel of water. A paper describing the apparatus and some of the results obtained with it was given at the Edinburgh meeting of the British Association in 1951 and is briefly summarized in Hardy & Bainbridge (1951*b*).

THE GENERAL PRINCIPLES OF THE METHOD

We will first describe the method and the working of the apparatus in general terms: this will be followed in a later section by a more detailed specification of its structure. A curved transparent tube of Perspex, of 2 in. by 1½ in. cross-section, is made to form a complete circle of 4 ft. diameter and is then mounted vertically to be rotated as a wheel. At one point on the rim there is an opening, which may be closed by a lid, sealed with vaseline and bolted down; through this, when it is turned to the top, the tube may be filled with water and the experimental animal put in. After sealing the opening, the wheel is turned so that the animal is halfway up one side. The apparatus, as will presently be explained, is provided with an overhead lighting as nearly as possible equivalent to under-water conditions; by a series of screens the intensity of this light may be varied from bright daylight to darkness. The animal is now free to move upwards or downwards; as it so moves through the water, the wheel is rotated carefully by hand so as to keep the animal opposite a fixed point outside the wheel, i.e. stationary in relation to the observer. It will be seen that the animal is now swimming in an endless column of water; so gradual is the curve, it can go on swimming *straight* up or down for hundreds of feet provided that the wheel is turned at a corresponding speed (see Text-fig. 1, p. 414). Inside the tube are small doors, some with weights and some with floats, which automatically open and close as the wheel goes round; they are always wide open on the side at which the animal is swimming, and so do not interfere with it, but are closed on the other side to ensure that the water turns exactly with the wheel and that the movement of this corresponds exactly with that of the animal.

The wheel is mounted in a frame. Fixed to its axle, so as to turn with it, is a pulley having a circumference exactly one-tenth that of the wheel itself; another similar pulley is mounted on the frame below it and a cord, kept at tension by a spring, passes round the two. On this cord is mounted a needle which records on the smoked drum of an electric kymograph. As the animal swims upwards the wheel is turned downwards and so the pulley system turns to move the needle upwards exactly one-tenth the distance moved by the animal. A graph at 1/10 vertical scale is thus traced on the drum, recording the upward and downward movements of the animal against a time base-line. If the wheel is skilfully turned so as to keep the moving animal always opposite the fixed position outside, its every little movement up or down is faithfully recorded for later analysis. It is in such cases, where the animal is jigging up and down, that the two kinds of doors referred to above are so valuable; they ensure that there is no lag of the water behind the movement of the wheel: it is moved as sharply as the wheel itself, because in *each* direction the thrust of the water at once meets a door closed tight against a stop. Because of this, together with quickness in following the movements of the animal, the tracing on the smoked paper can be an exact record of the animal's vertical migrational behaviour at a scale of 1/10.

The first experiments were made at the Plymouth Laboratory in a room having a skylight under which the wheel was mounted, the light being varied by a system of screens. It was realized that this was a poor representation of the illumination to be met with in the sea, so that a small glass house (a miniature 'greenhouse') was used for all the subsequent experiments which were carried out at Millport. The sides of it were covered with different thicknesses of paper, to give a graded light from above downwards, and the intensity of the light as a whole could be varied by a series of white, grey and black sheets which could be drawn over the roof and sides. Fixed to the frame, against the wheel and side by side with it, is one quadrant of an exactly similar Perspex tube: this was filled with water and received just the same illumination as the wheel itself. At the lower end of this quadrant, which is exactly opposite the point at which the animal is kept swimming, is fixed a photo-electric cell connected to a sensitive galvanometer which measures the light intensity; these values may be recorded at any moment for correlation with the vertical migrational behaviour of the animal in the tube. When working in darkness, a small red lamp, placed on one side of the tube, enabled the outline of the swimming animal to be kept silhouetted against a dim red disk of light; and thus the movement of the wheel could still be controlled to correspond with the animal's behaviour.

This description of the method will be sufficient to explain in general how the records were obtained and how a single animal can be followed whilst it swims through a column of water corresponding to a vertical height of maybe 200 or 300 ft. (as with the large euphausiacean *Meganyctiphanes norvegica*). The full details of the structure and arrangement of the apparatus will be given later (pp. 413–17).

LIMITATIONS OF THE METHOD

The ease of following an animal while swimming for such long vertical distances is achieved at the cost of certain disadvantages which must be recognized. There are three main limitations. First, conditions differ from those in nature in that as the animal swims upward or downward it does not experience the gradual reduction or increase in pressure that it would do in the sea. Some preliminary experiments have been done (Hardy & Bainbridge, 1951*a*) which appear to show that the behaviour of *Calanus finmarchicus* is not affected by pressure differences, although with decapod larvae a higher proportion of the population swims upward as the pressure is increased. It is unlikely that the small changes in pressure undergone as the animal swims a matter of a few feet will have an effect on its swimming behaviour, but clearly with forms like decapod larvae the *extent* of their migration in the apparatus may be very different from what it would be in the sea. Secondly, in the same way in the sea there would be a gradual increase or diminution in the light intensity as an animal swam up or down. This again does not occur in the apparatus, although it can be made to do so roughly either by adjusting screens overhead or by the use of artificial illumination with a dimming device; this, however, could not be in the continuous and gradual manner in which it is experienced in the sea. Thirdly, whilst it is swimming upward or downward in relation to the water passing it, the animal itself is not actually moving upwards because the wheel is turning against it to keep it stationary in relation to the outside world; consequently, if the wheel is turned exactly with the movement of the animal when it begins to swim, it will not experience any acceleration against gravity, i.e. the sensation we get when a lift starts to ascend or descend. This difference from nature will only occur at the moment of starting to rise or fall and will not be experienced once an animal is swimming at a more or less uniform speed. It is a difficulty which in practice is usually automatically overcome because the animal nearly always gets a slight start on the observer when it begins to swim, and the wheel is only subsequently turned to bring it opposite the pointer.

It may perhaps further be suggested that the movement of the animals is to some extent guided in an upward or downward direction by the proximity of the walls of the tube and that the path of swimming we have recorded is an artificial one. This might well be true if we were dealing with a large plankton animal such as a medusa which occupied a good deal of the cross-section of the tube; the plankton animals we are observing here however are so small in relation to the width of the tube that such a 'canalization' of movement does not occur. From time to time an upward swimming animal may converge on to and collide with the side of the tube; when this happens it may often dart away or turn a somersault so that the effect of the walls is rather to delay the upward swimming of an animal instead of guiding it in a vertical direction.

Animals which swim up diagonally will of course strike the sides more frequently than those which ascend more vertically.

Finally it may be thought that the wall of the tube may affect the swimming of the animals by offering a resistance to the movement of the water when they are swimming close against it. This indeed may have some effect when the animal is *very close* to the wall but, again because of the small size of the animal in relation to the cross-section of the tube, it will be against the wall only occasionally.

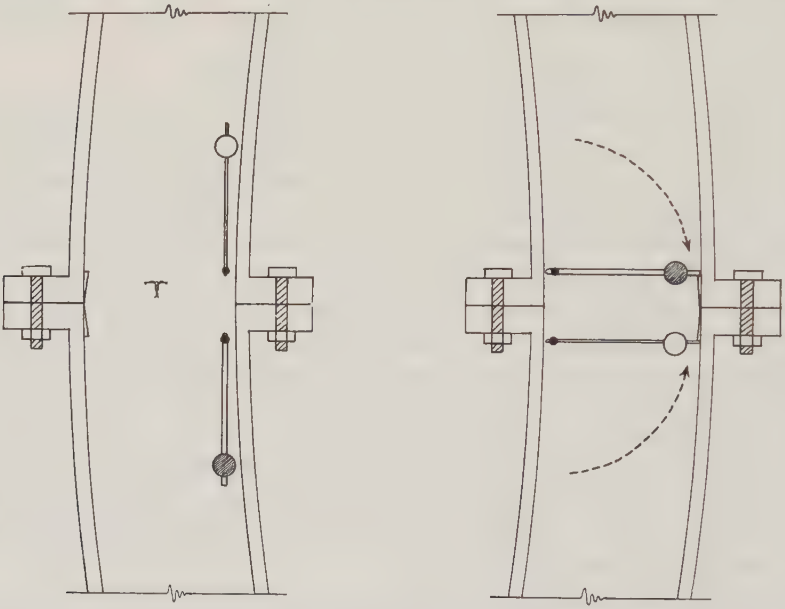
These various limitations must be kept in mind in the interpretation of results, but it is unlikely that they introduce a false representation either of the speed, duration or pattern of swimming behaviour of the animals considered. These factors will be referred to again in the discussion of results.

Construction of the Apparatus

The apparatus was constructed with the skilled assistance of Mr F. G. C. Ryder, to whom we are greatly indebted. The tube itself was built up of shaped sections of $\frac{1}{4}$ in. Perspex, the side pieces having been machined to shape and rebated to take the inner and outer pieces which were moulded by heating. The joints were made with chloroform and a solution of Perspex in chloroform and involved no bolting. There arc three sectors of tube and these are bolted together with six $2\frac{1}{2}$ in. 4 B.A. brass bolts through flanges of 1 in. Perspex attached to the ends of each. The internal dimensions of the tube so formed are $1\frac{1}{2} \times 2$ in., its length is 12 ft. and the diameter of the circle it produces roughly 4 ft.

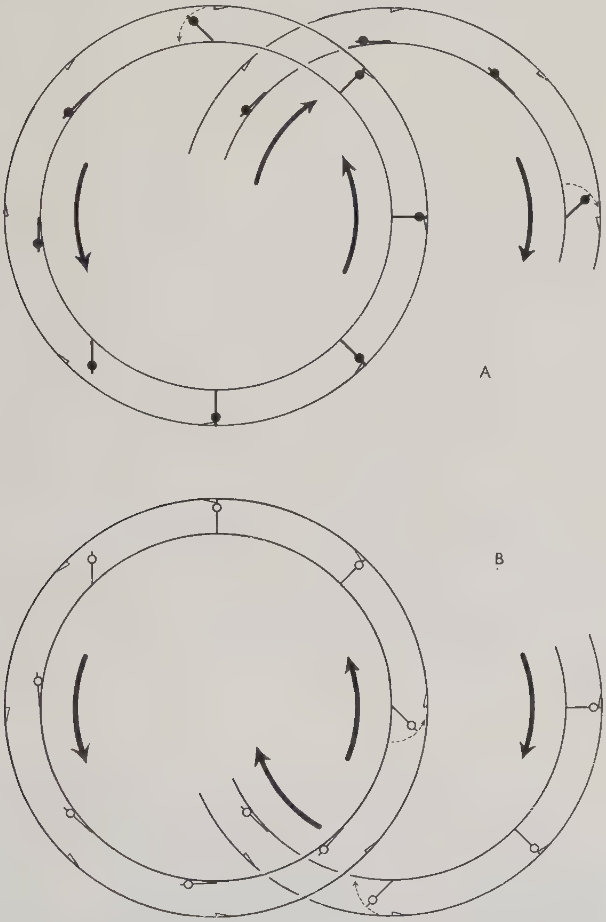
This circular tube is mounted on nine wooden spokes by means of Perspex clamps which allow a clearance of 1 in. between the tube and the wood. The spokes are attached to a boss and an axle which runs on two ball-races supported by a wooden stand (as shown in Pl. I A). Even when filled with water the wheel can be rotated with great ease.

A removable Perspex plate covers the filling opening of $1 \times \frac{3}{4}$ in. in the outer curved part of the tube. This closing plate is secured by four brass nuts and bolts, the bolts being embedded in a shaped piece of Perspex attached to the main curved section of the tube. The doors or valves, which ensure that the water turns exactly as does the wheel, are fitted in three pairs—one pair at each of the three pairs of flanges. They consist of rectangles of 1 mm thick Perspex cut to fit the cross-section of the tube exactly, and arranged to swing freely on an axle placed as near as possible to the inner (curved) wall of the tube. One door of each pair has a float made from a hollow cylinder of Perspex (of 1 cm diameter) attached close to its outer (free) edge and the other member has a weight consisting of a cylinder of lead encased in a hollow cylinder of Perspex attached in a similar position. The automatic action of these weights and floats ensures that the doors are always wide open on the side of the wheel facing the observer, where the animal is swimming, and closed on the opposite side (Text-fig. 1). Their cycle of opening and of closing against small wedge-shaped Perspex stops is shown in Text-fig. 2. Originally only one set, of weighted doors, was fitted. These suffice when the wheel is being turned steadily in an anticlockwise direction (when viewed from the right, as in the figures). When the direction of swimming changes, however, and the wheel has suddenly to be turned in the opposite direction, then the inertia of the water causes the doors on the side opposite the observer to be forced open and the movement of the wheel no longer bears a direct relationship to the movement of the animal. The inclusion of a second set of doors working with floats and closing against stops facing the opposite way ensures that there is always at least one door closed against a stop in whatever direction the wheel is turned. The efficiency of this method of locking the water to the wheel depends upon the elimination of leakage past the sides of the doors when the direction of rotation is being changed; and upon the free opening and closing of the doors by the forces of gravity. It is essential that the doors should be completely open opposite the observer in order to avoid obstructing the animal; in making the doors loose enough to do this, some slight leakage of water is necessarily entailed, but this is not sufficient to have an appreciable effect on the accuracy of the whole system.



Text-fig. 1. Diagram showing the opened and closed positions of the trapdoors at opposite sides of the wheel. A specimen of *C. finmarchicus* is drawn to scale swimming at the observation side.

The recording device consists, as already mentioned, of a large pulley wheel fitted rigidly to an extension of the axle of the wheel on the right-hand side with, below it, a second but free-running pulley of the same size fitted to the side of the wooden stand. The spring-tensioned cord connecting these two pulleys passes along a trackway of $\frac{1}{2}$ in. brass angle, 12 in. long, on the side of the pulleys away from the observer, and is connected to a slide which runs in the trackway and carries a recording needle. The pulley wheels were made to be precisely one-tenth of the diameter of the Perspex tube (taken from the centre of the tube at one side to the centre at the other) so that the needle moves through one-tenth of the distance swum by the animal and in the same



Text-fig. 2. Diagrams showing the cycle of the opening and closing of (A) the weighted doors, and (B) the buoyant doors according to the way the wheel is turned—either clockwise or anticlockwise.

direction; in doing so it impinges on an electric kymograph drum carrying smoked paper. By this means a graph representing the movement of the animal is obtained, with time along the horizontal axis and distance swum vertically along the vertical axis. Should the animal swim persistently in one direction then the pointer comes sooner or later to the top or the bottom of the paper. Just before it does so, however, the slide completes an electric circuit by closing a pair of spring contacts (seen in Pl. II B) and so flashes a corresponding warning light (in foreground of Pl. I A); the slide and pointer are then at once returned to the opposite end of the trackway by pulling sharply on the tension spring. This action draws on the recording drum a straight vertical line which is readily distinguished from the more irregular and sloping lines resulting from the movement of the animal. The warning lights are particularly valuable when working in darkness.

Two factors of prime importance in a study of the effect of various environmental changes on the movements of the animals used are the intensity and the directional nature of the light falling on the animal. Every effort was made to ensure that these were as close as possible to those found in nature. The place in the wheel usually occupied by the animal was arranged to be as close as possible to the centre of the base of a rough dome of light. This was done by housing the complete apparatus in the greenhouse which measured 5 ft. 9 in. by 3 ft. 10 in. by 6 ft. 6 in. high. The lower 4 ft. 6 in. of the walls was completely blacked out and the remaining part was made to transmit a gradation of light intensity by sticking diminishing thicknesses of tissue paper on to the glass. The roof was left clear but a white linen sheet was thrown over the whole house to bring the intensity down to one comparable with those found in the sea. The greenhouse is shown in Pl. I B, but the paper screening has been removed to show the position of the wheel inside the house.

The directional nature of the light was kept constant throughout the investigations, but the absolute intensity was either varied artificially or allowed to vary naturally. The former was effected by drawing grey and black sheets over the house; and the latter resulted from the normal passage of clouds over the sun. In order to obtain a measure of the actual intensity falling on the animal inside the wheel the extra stationary sector of tube was mounted alongside it as shown in Pl. II A. This sector was one-quarter of the whole circle and its lower end, closed with a 1 mm thick Perspex plate, was arranged to be directly opposite the position occupied by the animal. It was completely

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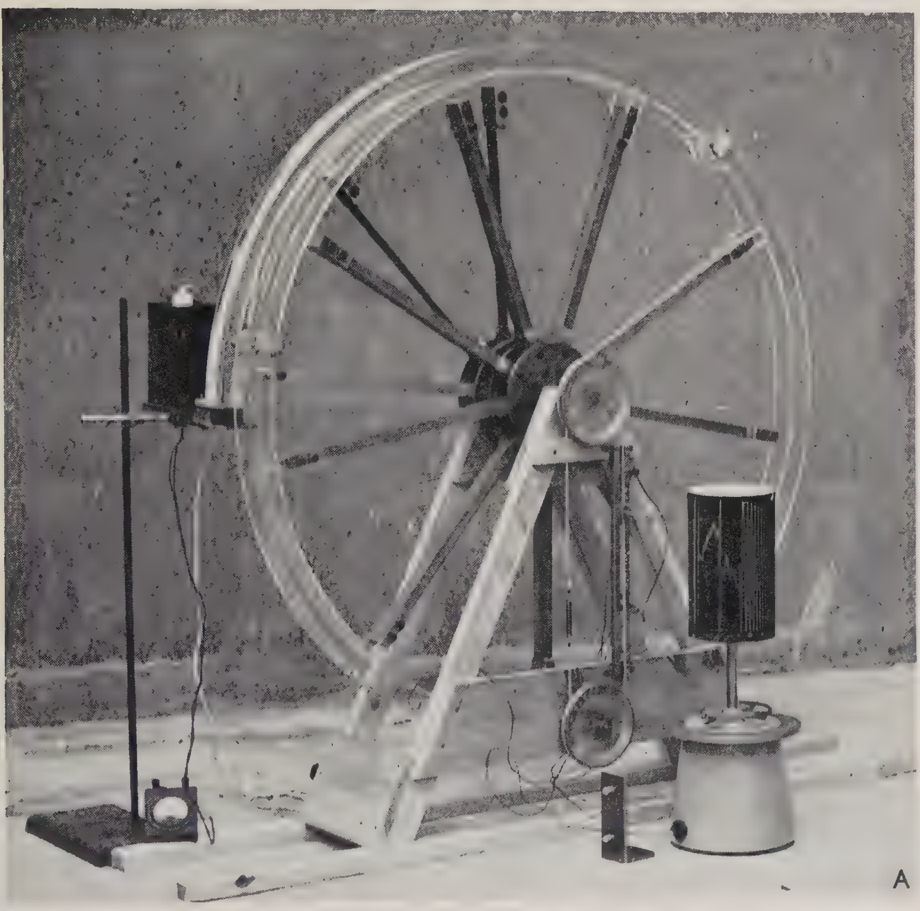
EXPLANATION OF PLATE I

A. General view of the wheel apparatus for the study of the vertical migration of plankton animals.

B. The apparatus mounted in the small greenhouse; the paper screening designed to give a gradation of light intensity has been removed to show the position of the wheel.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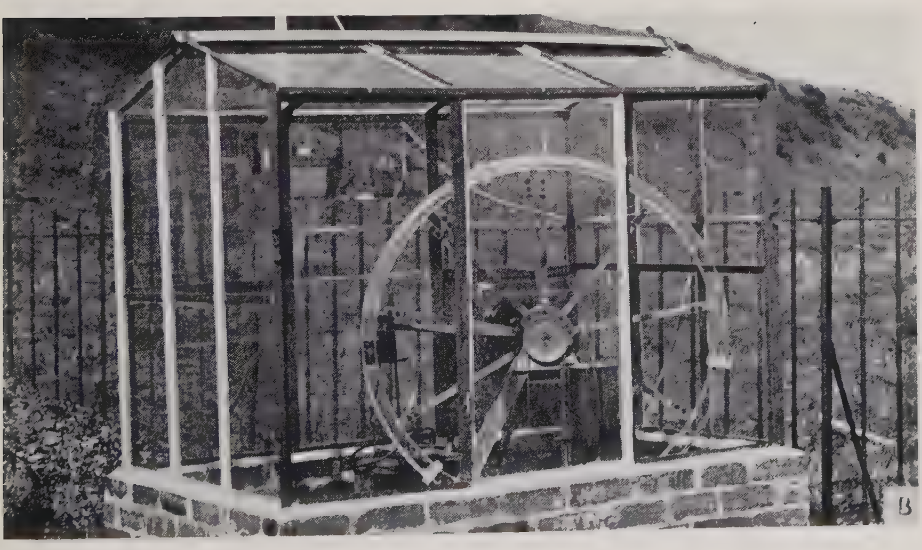
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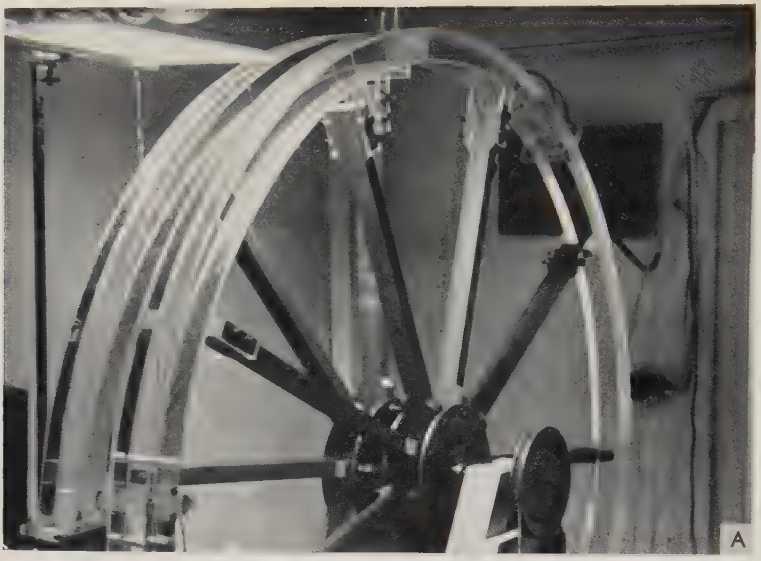
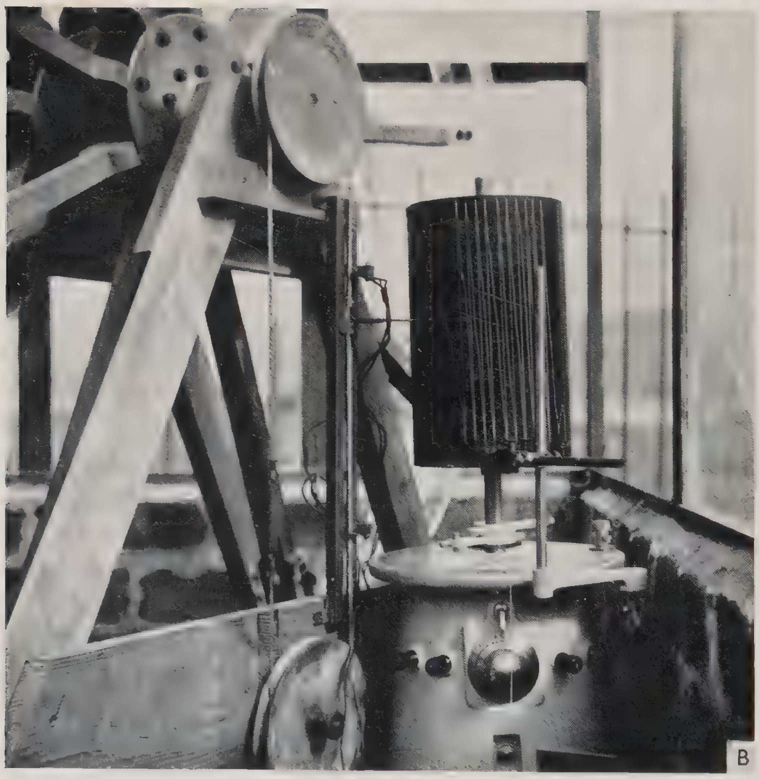

A


B

filled with water through a funnel at the top and an Electro-Selenium Photocell was bolted immediately below the Perspex plate and pointed directly upwards. The current produced by this photocell was measured at regular intervals throughout the course of an experiment with a galvanometer, and the current value was used to calculate the light intensity falling on the cell. This is assumed equal to that falling on the animal inside the wheel, because the stationary sector is identical in every respect to a sector of the wheel itself.

In order to use a photocell such as the E.E.L. cell used here for measurement of light intensities, it is necessary for it to be calibrated against a standard cell. This was most kindly and thoroughly done for us by Dr W. R. G. Atkins, F.R.S., of Plymouth, who also gave us valuable advice on the use of cells and circuits. The arrangement finally adopted consisted of an E.E.L. selenium rectifier cell mounted under an opal flashed glass and a Cambridge Unipivot type L galvanometer no. 65542. These were connected with shunts of various value depending upon the light intensity at the time. The galvanometer reads up to 24 μA, which is equivalent to about 250 Lux of incident light. For light intensities higher than this it is necessary to use a shunt in order to reduce the amount of current passing through the galvanometer. Shunts of 100, 40, and 20 Ω were employed and, with the latter, light intensities up to about 10 kilolux could be measured. Unfortunately the current produced by this type of cell varies according to the resistance in the external circuit, and the cell had therefore to be calibrated separately for each shunt. When this was done graphs of scale reading of galvanometer against light intensity were drawn and the conversion of any subsequent experimental reading simply entailed inspection of these.

Use of a greenhouse to obtain the required type of illumination introduced the problem of keeping the apparatus cool. This was accomplished fairly satisfactorily by fitting a Vent Axia fan to draw out the warm air and by watering the roof at intervals with cold water from a watering can. Evaporation from the damp sheet then reduced the temperature considerably.

Examples of Records

The results will be understood more easily if they are introduced by showing and describing a few examples of the records made with the apparatus. Text-fig. 3 shows the first one to be made: that taken at Plymouth on

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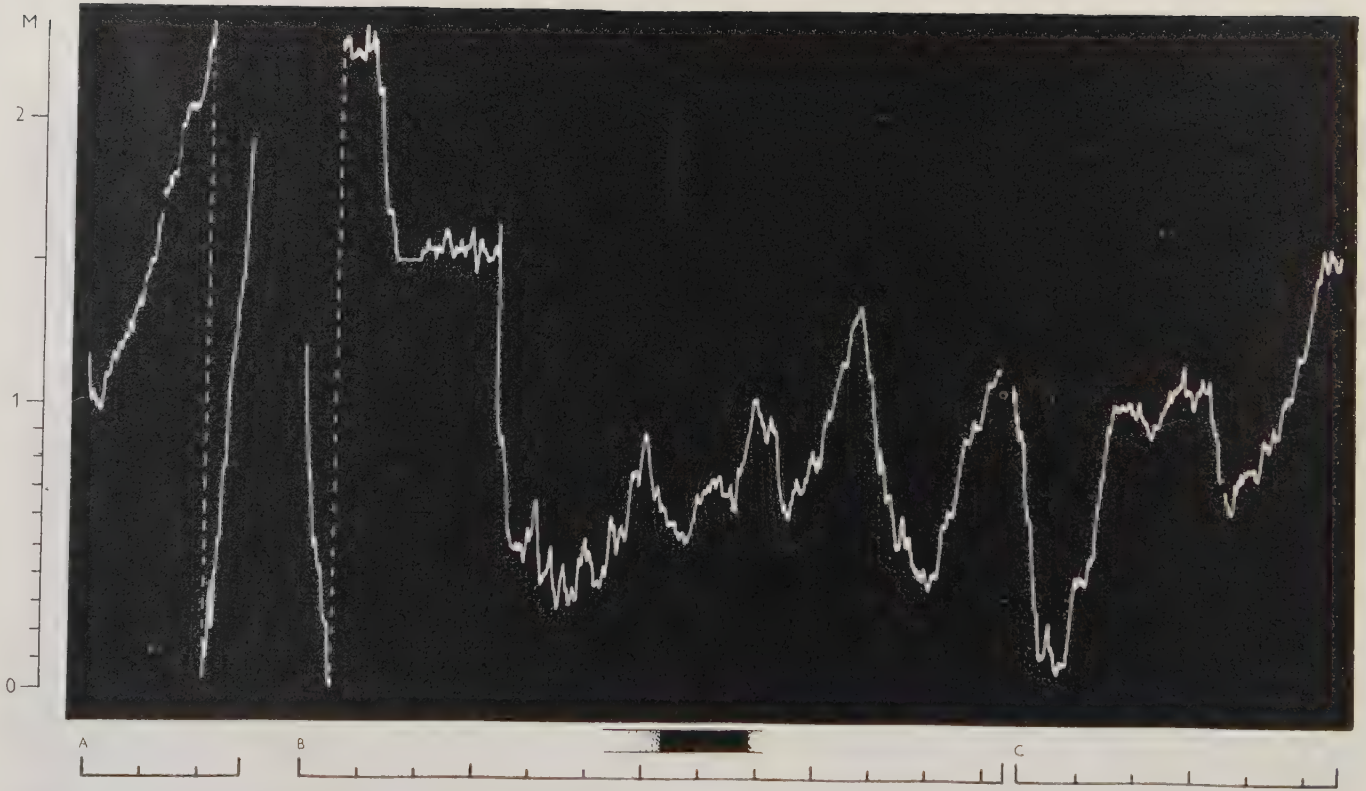
EXPLANATION OF PLATE II

A. Detail of the upper part of the wheel to show the extra parallel quadrant for the measurement of light intensity; note also the buoyant and weighted doors in the open position inside the wheel on either side of the sectional junction flanges at the point nearest the camera.

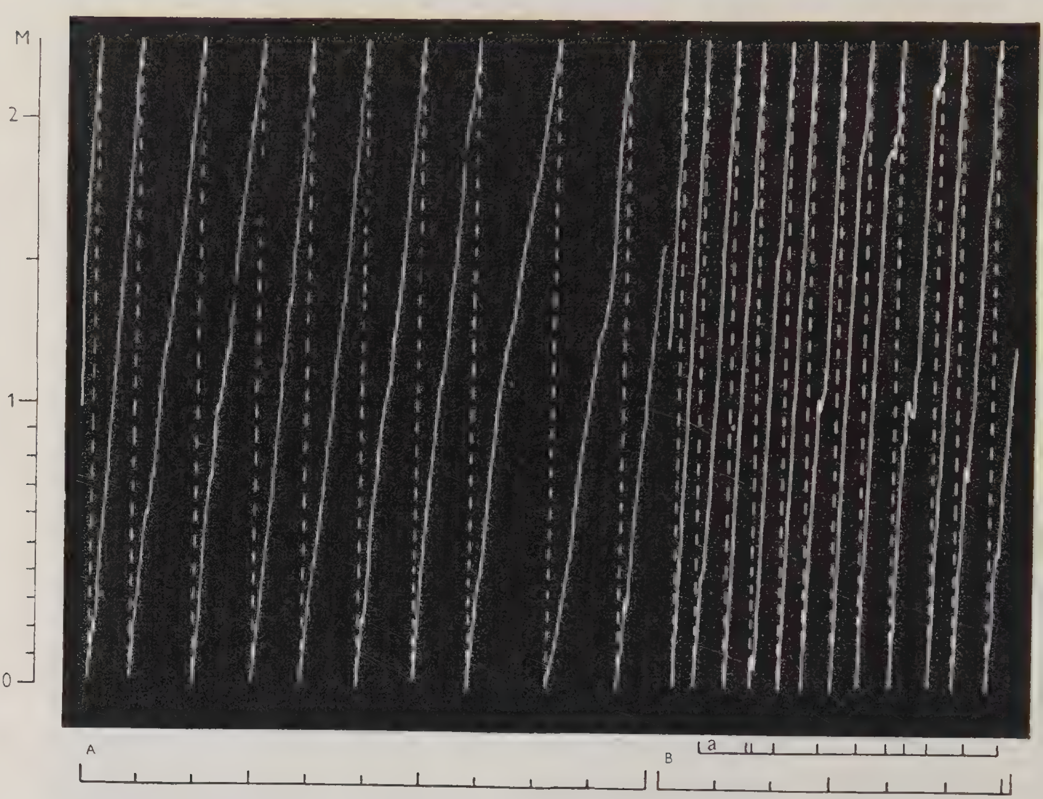
B. Details of the recording mechanism.



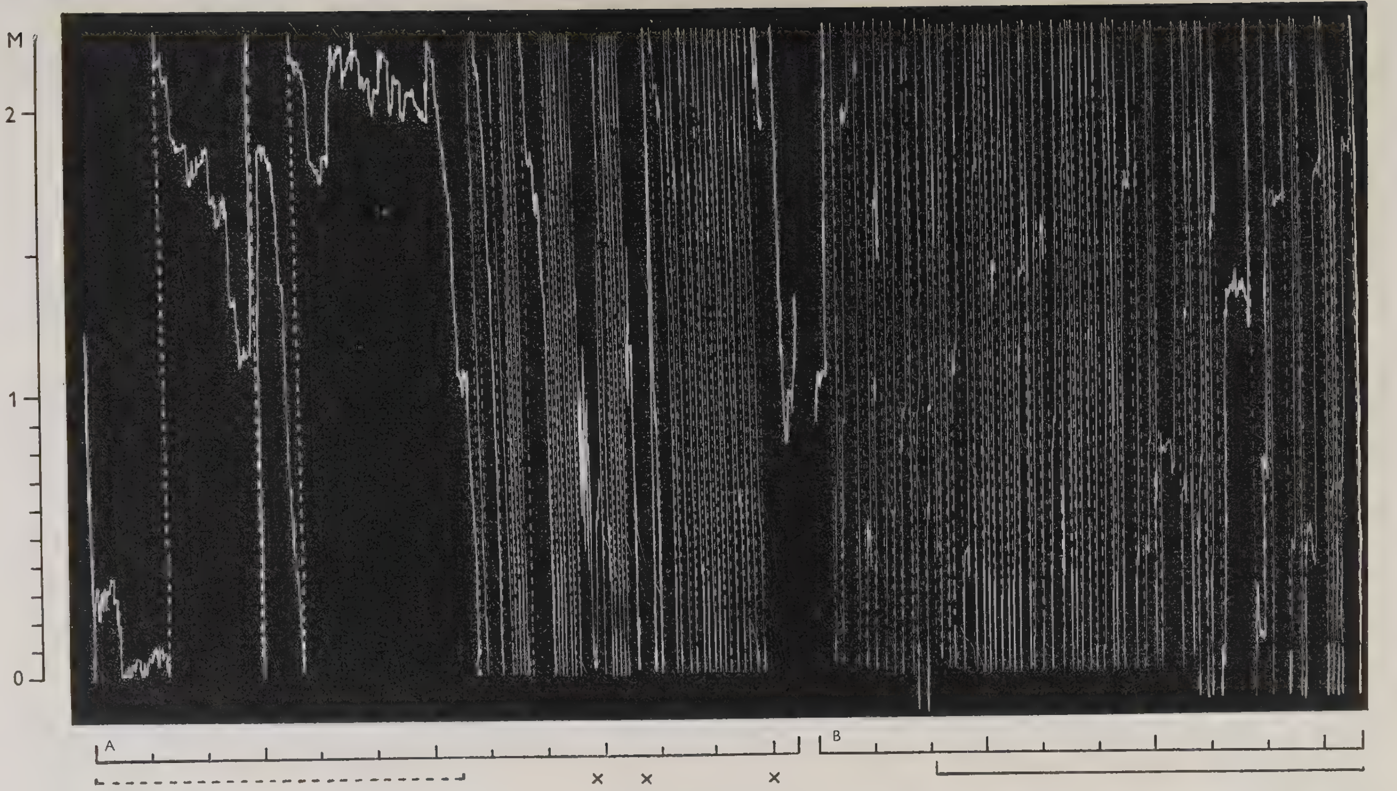
Text-fig. 3. Reproduction of the kymograph tracing obtained in record no. 1 experimenting with *C. finmarchicus*. A, a specimen observed from 18.05 to 18.40 h on 15 March 1950; B, a second specimen from 10.51 to 12.55 h on 16 March 1950; C, the same specimen from 14.54 to 15.51 h on the same day, the water in the wheel having been enriched with diatoms and flagellates. The time scale is divided into 10 min periods (except for period A when the kymograph was rotating more slowly). The blackened and dotted rectangle in B indicates a period of partial and complete blacking out. For full description see text opposite.

15–16 March 1950 with the copepod *Calanus finmarchicus*. It begins with a 35 min period from 18.05 to 18.40 h. The smoked paper, from the rotating kymograph drum, is moving to the left at a rate of 2 mm/min, and, as already explained, the graph traced by the recording needle represents exactly one-tenth of the distance travelled by the animal. In reproduction the graph is further reduced, but a scale shows the *actual* distance travelled in metres. During this first 35 min the *Calanus*, while jigging up and down to a certain extent, is moving upwards with a gradual acceleration; it climbs 0·8 m in the first half of this period and 2·32 m in the second half. The broken vertical lines represent where the recording needle has been moved from the top to the bottom of the paper, or *vice versa*. The second part of the record is made from 10.51 to 12.55 h on the following day. *Calanus*, a different specimen, now descends rapidly 1·25 m then jigs up and down for 10 min, descends again 0·75 m, remains quite stationary for 5 min and then jigs again for another 18 min before diving down, swimming head first, for another metre. From now until this part of the record was stopped at 12.55 the *Calanus* rises and falls with little bursts of active swimming followed by short intervals of passive sinking or, occasionally, downward swimming; for alternating periods the movement is more upward than downward and *vice versa*, producing the main peaks and troughs shown on the graph. Halfway through this period the light from above was gradually blacked out to complete darkness for 15 min and then gradually restored again, as shown in the figure; this change in light intensity appeared to have no effect upon the behaviour of the animal. The last part of the record was taken for just under an hour, 14.54 to 15.51 h, with the *Calanus* in water rich in diatoms and flagellates (added from cultures): it swims downwards at first for nearly 1 m, then upwards, jigs for a time and then downwards again for 0·5 m followed by an irregular upward movement.

The second example is record no. 4 made, also at Plymouth, on 30 March 1950 and shown in Text-fig. 4. Here from 11.30 to 13.10 h we are following the movement of a group of *Balanus* nauplii which kept together as a cluster and swam upwards with great regularity. We are not following one individual all the time, for in the cluster it was difficult to be certain which one was which, as they swam near each other and passed and repassed one another. But because they kept so well together the graph does in fact closely represent the path taken by one individual. The speed of swimming and the energy displayed are surely remarkable for such small animals; for, during the 1 h 40 min they were observed, they never stopped swimming and averaged a climb of 14·9 m/h. The speed of ascent of the cluster as a whole varied somewhat as is indicated by the distances between the vertical broken lines: in each such interval the cluster has climbed approximately 2·25 m and the varying times in minutes taken for this distance are 7·5, 11, 10·5, 8·5, 9·5, 9·5, 9·5, 13·5, and 12. The wheel was now stopped and without emptying out the water a small medusa of about 1 cm diameter, probably a late ephyra stage of *Aurelia*, was added; during the second part of the record, from 13.52 to 14.54 h, we followed this medusa and not the nauplii which remained in the water with it. The medusa, like the nauplii, was swimming steadily upwards but at a speed of 27·1 m/h, i.e. nearly twice as fast. Since we were now keeping pace with the medusa, the nauplii were carried round with the wheel and from time to time were overtaken by the medusa; each time this occurred the medusa stopped for a moment and with a few deft movements of the manubrium snatched up one or two nauplii before proceeding on its way. These pauses are clearly shown on the record as marked below the graph. The position of each nauplius must have been determined by the combined action of the tentaculocysts which presumably were picking up the vibrations in the water made by its swimming movements; each movement of the



Text-fig. 4. Kymograph tracing from record no. 4. A, showing the upward migration of *Balanus* nauplii from 11.30 to 13.10 h; B, the upward migration of a small medusa from 13.52 to 14.54 h. The time scale is divided into 10 min periods. The line at *a* indicates pauses for the eating of nauplii: see text on this page.



Text-fig. 5. Kymograph tracing from record no. 10. A, showing a medium-sized *Meganyctiphanes norvegica* observed from 18.45 to 20.50 h; B, a large *M. norvegica* observed from 22.11 to 23.44 h. The dotted line under A indicates the period during which the ends of the greenhouse were shaded. The crosses indicate occasions when the animal was trapped behind a door. The unbroken line under B indicates the period during which the animal's photophores could be seen glowing. The time scale is divided into 10 min periods. See text, p. 422.

manubrium appeared to be made directly at the prey just as if it was a hand snatching up food in passing.

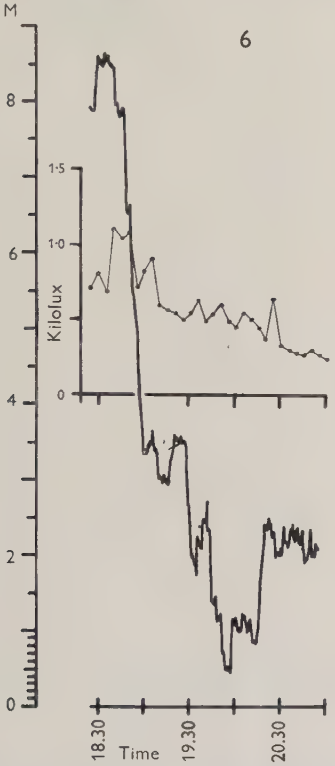
One other example, Text-fig. 5, will be given to show an animal swimming at considerable speed: record no. 10 made in the small greenhouse at the Millport Laboratory on 14 June 1950 with the euphausiacean *Meganyctiphanes norvegica*. The record starts at 18.45 h with a medium-sized animal and the greenhouse has each end shaded with a black cloth. At first the animal has an irregular up-and-down movement lasting about half an hour but with a downward tendency, then it swims rapidly upwards for about 3 m and down again for 3 m, after which it swims irregularly again for another 20 min or so until the dark sheets shading the ends of the greenhouse are removed leaving only the white sheet over the whole top. As soon as this change in lighting is made the animal swims down at great speed and continues to do so until the end of this first part of the record at 20.50 h, except at the points marked with an *X*; at these points the animal, in its headlong downward dash, has accidentally swum behind one of the doors of the wheel and some time has elapsed before it can be got out of this position by jerking the wheel to and fro. This jerking of the wheel is shown on the graph at the first *X*, but later the recording needle has been lifted off the drum whilst these artificial movements were made. The second part of the record was made on the same evening from 22.11 to 23.44 with a different and fully grown specimen of *M. norvegica*. This animal, except for short pauses which become more frequent and of longer duration towards the end, swam rapidly upwards covering a distance of approximately 132 m in the $1\frac{1}{2}$ h. During part of the upward swimming, as indicated on the chart, the photophores could be seen to be shining.

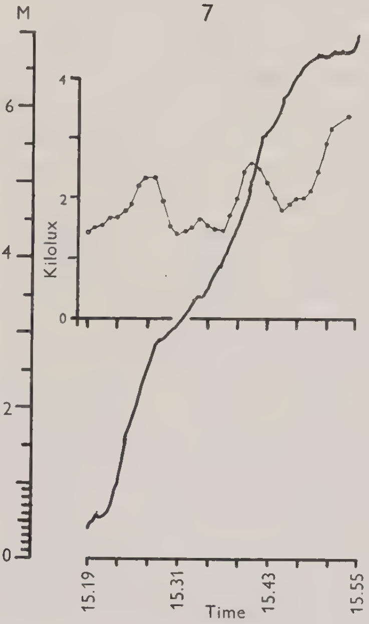
Survey of Results

Altogether fifty records were made with the apparatus, each averaging about 3 h duration. It is not proposed to reproduce and describe all of these but rather to select those which best illustrate the various kinds of observations made and the scope of the work as a whole. The kymograph speeds have varied from 1·5 mm/min in some records to 5 mm/min in others; a time scale for each record is provided below each tracing reproduced.

Types of swimming behaviour

While the automatic recording device is making a permanent record of a migration it is possible to make sustained observations on the behaviour of the animal employed. It can then be seen that some animals swim in a characteristic and recognizable fashion; and this movement may be so distinctive as to impart a characteristic pattern to the smoked drum record. An example of such a pattern may be seen in Text-fig. 6 (record no. 28) which shows the swimming typical of *Labidocera*. This consists of fairly long bursts of steady swimming separated by roughly equally long periods of jigging up and down. In contrast, record no. 22 shows, in Text-fig. 7, the steadier swimming of *Centropages* and record no. 10 (Text-fig. 5) the steady and much faster swimming of *Meganyctiphanes*. A trace typical of a zoea larva is record no. 24, in Text-fig. 8, which shows steady swimming to be possible but also that this may be replaced by an indeterminate bobbing up and down which gives no resultant movement in either direction.

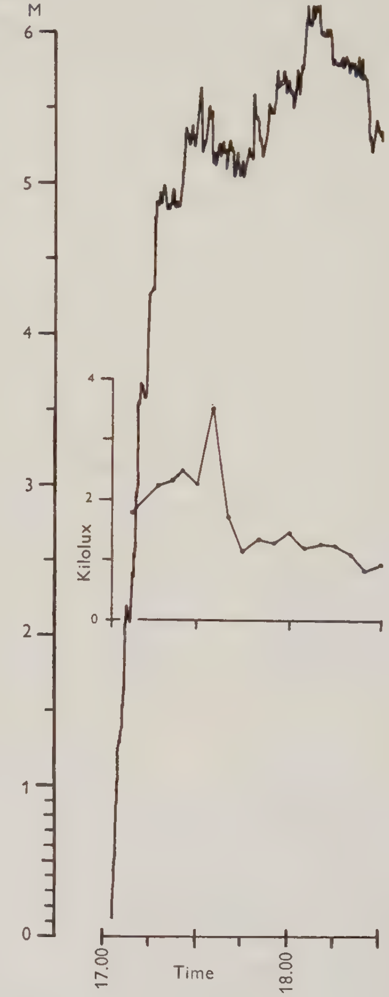




Text-fig. 6. Continuous tracing from part of kymograph record no. 28, showing the behaviour of *Labidocera wollastoni* ♂, together with a graph of changes in light intensity (25 July 1950).

Text-fig. 7. The same of record no. 22, showing the behaviour of *Centropages* sp. ♀, but with different scales for time and light intensity (20 July 1950).

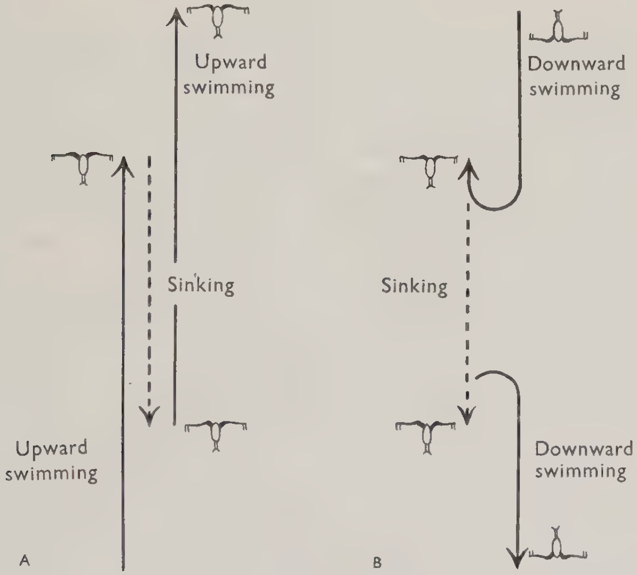
Generally, amongst the animals used, two distinct types of movement can be distinguished. The first of these is an up-and-down swimming which appears to be quite independent of the nature of the tube; and the second is one with a greater horizontal component which may in fact sometimes entirely replace up-and-down movement. The first type seems to embrace three subdivisions—the direct, the indirect, and the 'hop-and-sink'. The larger copepods such as *Calanus*, *Labidocera* and *Temora* swim up and down in a direct manner. Some of the smaller copepods such as *Acartia* and *Paracalanus* swim up and down with an indirect motion; i.e. while their resultant movement is in an upward or downward direction they follow an irregular zigzag path, still keeping clear of the sides of the tube but not moving *directly* up and down. Movement of the larger copepods is generally as described but is not invariably so. They may swim in the steady fashion for long distances but may also progress with a 'hop-and-sink' movement, when short bursts of upward swimming alternate with periods of passive sinking. A most important observation is that the downward migrations are almost invariably a head-first swimming downwards and not a passive sinking. The counterpart of the 'hop-and-sink' may take place in a downward direction when it is complicated by the natural tendency of the animal to float in an upward position. This results in each burst of downwards swimming being followed by a curving loop of the animal into an upward position; this is followed by a period of passive sinking (tail first) and this in turn by a resumption of swimming and a curve over to go head-first down again. These two forms of 'hop-and-sink' behaviour are compared in Text-fig. 9.



Text-fig. 8. Continuous tracing from part of record no. 24 showing the behaviour of a zoea larva, together with a graph of changes in light intensity (21 July 1950).

The second type of swimming is indulged in by the euphausiids, decapod larvae, and by the larger copepod *Euchaeta*. These forms, while often making straight up-and-down excursions of the greatest length and speed, may also swim horizontally into the sides of the tube. When they do this it is impossible to make a recording of them although of course, if they were in the sea, they would be moving horizontally or at a large angle to the vertical.

Besides these large-scale movements of up-and-down or sideways swimming it is possible to detect by eye smaller-scale movements which are not recorded on the trace. Amongst the copepods it is clear that the steady and general type of propulsion involves the thoracic appendages and this forms the basis of the 'hop-and-sink' movement too. For short and much more rapid leaps, both violent movement of the antennae and flexing of the abdomen appear to be employed. Occasionally a violent caper may be indulged in when an animal leaps about in all directions with so great speed that it is not possible to determine which appendages are used.



Text-fig. 9. Diagrams illustrating the two types of 'hop-and sink' behaviour of *Calanus finmarchicus* during (A) upward swimming, and (B) downward swimming.

The underwater observations reported in Bainbridge (1952, 1953) were undertaken expressly to determine whether the swimming observed in apparatus of this type is similar to that occurring in the sea. The results reported make clear that it is so, and in general the observations made on animals in the wheel may be taken as applying also to behaviour in the sea. In particular the direct, the indirect, and the 'hop-and-sink' modes of up-and-down swimming have been observed in the sea, as has some amount of horizontal swimming and occasionally the violent caper mentioned above. The close parallel between all these movements in the wheel and in the sea makes it clear that reliance may be placed on the results and serves to refute the criticism that the 'canalizing' effect of the tube must make behaviour in the wheel unnatural.

None of the animals which have been fitted into the above categories must be taken as moving solely in the manner described there. All the species mentioned have been observed to swim, at one time or another, in several or all of the ways described. Behaviour is both individualistic and variable. The account (p. 420) of the change in swimming of the medusa in the presence of *Balanus* nauplii serves to demonstrate both how swimming movements can be altered to suit particular circumstances and also how useful the wheel may be in making such observations.

Speeds of swimming

In addition to giving a record of the different types of swimming behaviour, the apparatus enables us to measure the actual distances various animals are capable of swimming upwards and downwards in given periods of time, and so to compare their speeds of swimming. The experiments take a long time to set up and perform so that at present our results must be regarded only as an indication of the kind of speeds attainable by certain organisms; many more records must be taken before it will be possible to state the limits to their speeds of vertical swimming. For only a few organisms have we records of both upward and downward swimming. So far some species would only swim up in the apparatus and others only down. Nevertheless, for the first time we are able to show the actual distances which may be travelled vertically in periods of 30 min or 1 h by a number of different species. This information is most easily presented in the form of a table. In Table I, as far as possible for each animal, is shown the maximum distance travelled in a short period of 2 min (or sometimes 1 min), in 30 min, and in 1 h, together with the speed maintained for these periods expressed in metres per hour. There are only two animals for which we have sufficient records to enable us to compare the speeds of upward and downward swimming: the copepod *Calanus finmarchicus* and the euphausiacean *Meganyctiphanes norvegica*. For a short period of 2 min *Calanus* can swim upwards at a speed of 66 m/h and downwards at 107 m/h; maintained for a whole hour the speeds are only 15 and 47 m/h respectively. The copepod *Centropages* can climb 30 m in an hour. *Meganyctiphanes* can swim upwards at a speed 173 m/h for 2 min and downwards at 215 m/h for the same period; for a whole hour the respective speeds are 92·8 and 128·8 m/h. The remarkable speeds attained by so small a creature as a *Balanus* nauplius, as well as its powers of prolonged swimming, have already been commented on (p. 419).

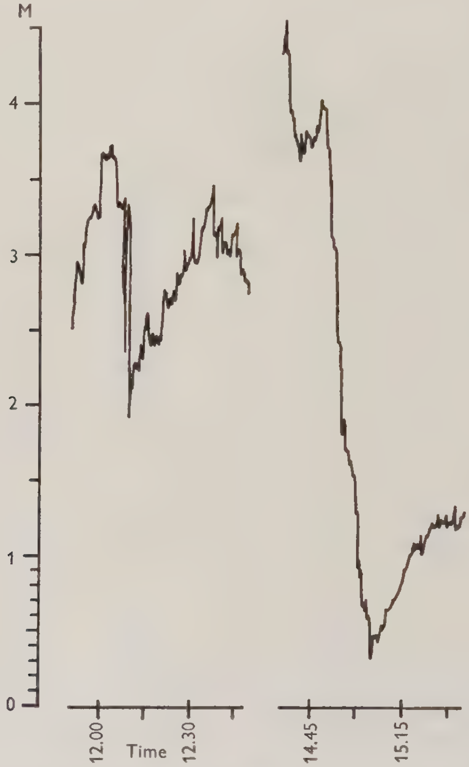
Differences between animals

There are in general considerable differences in behaviour between animals which might be expected to react similarly. While the reaction—positive or negative—towards light may usually be uniform within a group of apparently identical animals caught at the same time, their speeds of swimming will differ

Table I. Distances Travelled and Speeds of Vertical Swimming of Various Plankton Animals over Different Periods of Time

	Up			Down		
	Duration (min)	Distance (m)	Speed (m/h)	Duration (min)	Distance (m)	Speed (m/h)
Calanus finmarchicus	2	2·2	66	2	3·57	107
	30	17·8	35·6	30	29·9	59·8
	60	15	15	60	47	47
Euchaeta norvegica	—	—	—	2	4·52	135·6
	—	—	—	30	41·25	82·5
	—	—	—	60	70·4	70·4
Labidocera wollastoni	2	0·99	29·7	2	0·78	23·4
	—	—	—	20	7·97	23·9
Centropages sp.	2	1·82	54·6	2	0·7	21·0
	30	16·1	32·2	—	—	—
	60	30·6	30·6	—	—	—
Temora longicornis	2	0·63	18·9	2	0·57	17·1
	30	4·47	8·94	—	—	—
	60	5·13	5·13	—	—	—
Paracalanus parvus	2	1·09	32·7	—	—	—
	30	2·14	4·28	—	—	—
	60	3·24	3·24	—	—	—
Acartia clausi	2	1·12	33·6	2	0·57	17·1
	30	4·43	8·86	8	1·61	12·1
	60	8·81	8·81	—	—	—
Oithona similis	2	0·8	24·0	—	—	—
	30	3·96	7·92	—	—	—
Bopyrid larva	—	—	—	2	1·75	52·5
	—	—	—	30	10·95	21·9
Calanus nauplius	2·5	0·185	4·44	—	—	—
Balanus nauplius	2	0·75	22·5	—	—	—
	30	7·63	15·26	—	—	—
	60	14·9	14·9	—	—	—
Zoea (Brachyura)	1	1·17	70·2	—	—	—
	2	1·95	58·5	—	—	—
	10	4·89	29·34	—	—	—
Meganyctiphanes norvegica	2	5·77	173·1	2	7·17	215·1
	30	62·21	124·4	30	68·2	136·4
	60	92·8	92·8	60	128·8	128·8
Thysanoessa inermis	—	—	—	2	4·15	124·5
	—	—	—	30	19·5	39·0
	—	—	—	60	40·4	40·4
Tomopteris	—	—	—	2	6·84	205·2
	—	—	—	28	47·46	101·7
Sagitta elegans (small, ½ in)	1	0·44	26·4	2	0·3	9
	—	—	—	15	1·17	4·68
Rossia macrosoma	—	—	—	2	4·58	137·4
	—	—	—	30	44·65	89·3
Small medusa late Ephyra (1·0 cm diam.)	—	—	—	2	1·29	38·7
	—	—	—	30	15·06	30·12
	—	—	—	60	27·1	27·1

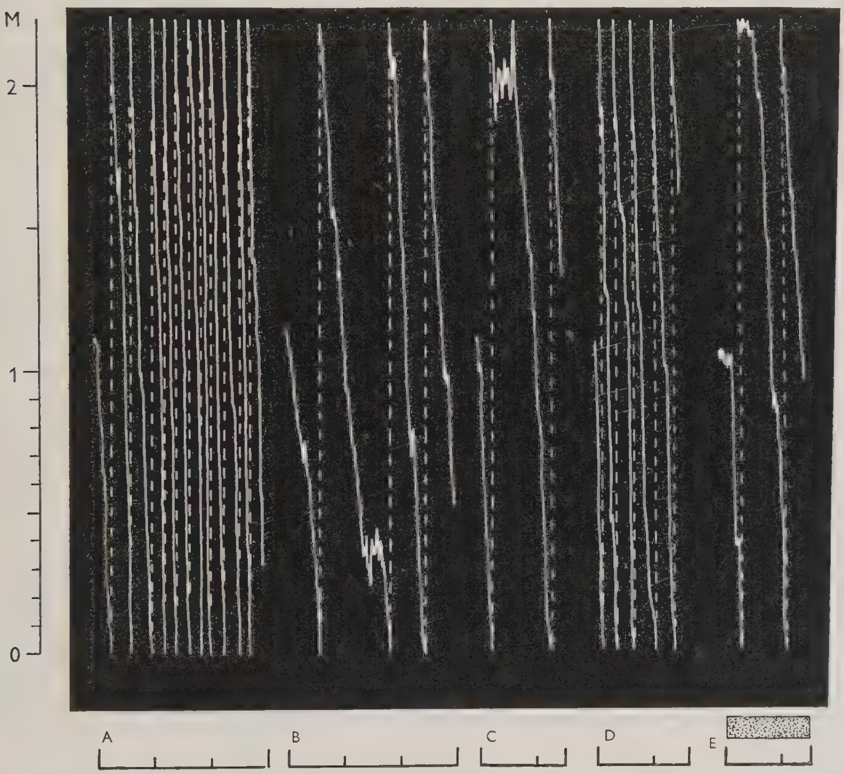
greatly and one or two will even behave in the opposite manner. In four separate experiments with four similar *Calanus* females each caught at 10.30 h and experimented with at 11.00 h on similar days, speeds for swimming down of 50·8, 6·7, 50·8 and 13·5 m/h were obtained. On another occasion, of two *Calanus* females caught at the surface at the same time, the first experimented with showed a somewhat uncertain, but slightly downward, movement from 11.52 to 20.45 h, finally swimming down at 13·7 m/h just before 20.45; the second, having been kept in a breffit during the day, was put in the wheel at



Text-fig. 10. Continuous tracings from kymograph record no. 46 showing the behaviour of *Calanus* in complete darkness (10 July 1951).

20.55 h and swam up consistently at 18·6 m/h. Even the same animal may show differences under conditions which must be considered identical. Record no. 46 shows, in Text-fig. 10, the swimming of a *Calanus* on two occasions separated by 2 hours. In one there is a slight upward resultant and in the other a downward one. At both times the greenhouse was completely blacked out. Record no. 43, which is discussed in the light intensity section (see Text-fig. 23 on p. 442), shows, further, how inconsistent the reaction of an individual (*Cyclops*) may be to some regulated stimulus such as partial blacking out.

This occurrence of variation where uniformity might be expected makes all the more difficult an interpretation of those cases in which differences might be expected. Three recordings were made with *Calanus* taken from the surface and from 60 m. These were members of the spring brood which, in the Clyde area, is often to be found partly or almost wholly up at the surface. In the first example, record no. 40 given in Text-fig. 11, a *Calanus* male taken at 60 m swam down at 51·3 m/h and in a second test at 39·1 m/h; a *Calanus* female taken at the surface also swam down but at 14·9 and 17·2 m/h in separate tests. In a second example, not illustrated, a female from 60 m



Text-fig. 11. Reproduction of kymograph tracing from record no. 40. A, *C. finmarchicus* ♂ from 60 m depth, observed from 11.25 to 11.55 h, 10 May 1951; B, *C. finmarchicus* ♀ from surface, observed from 12.10 to 12.40 h, 10 May 1951; C, same *Calanus* as in B but observed from 11.40 to 11.55 h on 11 May 1951; D, same *Calanus* as in A but observed from 11.57 to 12.12 h, 11 May 1951; and E, the same *Calanus* from 12.25 to 12.40 h. The stippled rectangle in E indicates partial blacking out of the greenhouse. The time scale is divided into 10 min periods. See text below.

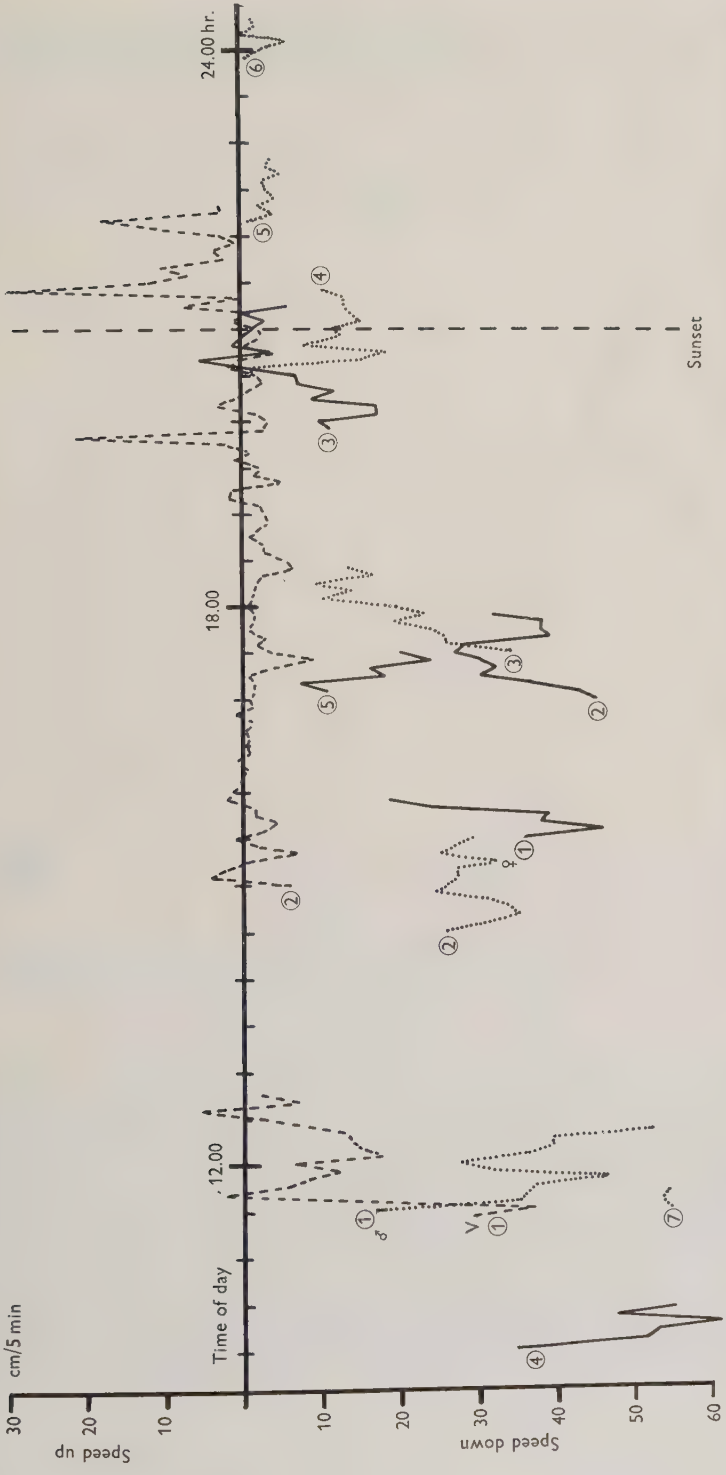
swam down at 1·4 m/h while one from the surface swam up at 35·6 m/h. In both these cases there is an understandable difference between the two types of animal, but in a third example the behaviour of stage V animals both from the surface and from 60 m was identical and consisted in swimming down at about 4 m/h. A further anomaly is revealed if the 12.10–12.40 period of swimming down in record no. 40 is compared with that of a precisely similar animal 2 days later from 12.10 to 12.25 in record no. 41 (p. 445); here a *Calanus* from the surface is found swimming up at 6 m/h. The light intensity on both these occasions was in the region of 6 kilolux. These differences are puzzling, but the possibility, suggested by Bainbridge (1952), must be borne in mind of there being no fundamental difference between the so-called 'surface' and 'deep' forms of *Calanus*.

Changes in behaviour with time

A series of experiments was performed with *C. finmarchicus* in which records were made each day for 1 h periods at 11.30, 14.30, 17.30 and 20.30 h, and also occasionally at intermediate times and later than 21.30 h. The animals used were caught immediately prior to testing and were taken from about 40 m depth. The aim of these experiments was to see if any change in behaviour occurred as the time for normal vertical migration in the sea approached. A change was generally clearly visible. When first caught the animals would swim down rapidly, but at later periods the speed would become less and less and sometimes an upward migration would eventually take place. There was much variation in the results but never was there an upward migration early in the day or a rapid downward one late in the day. This change of behaviour must be due either to the alteration in light intensity or to an alteration in the reaction of the animals rather than to their becoming moribund or adapted to the circumstances of their confinement, because, when it was possible to test the same animal on the next morning, the same vigorous downward swimming characteristic of a newly caught specimen was indulged in.

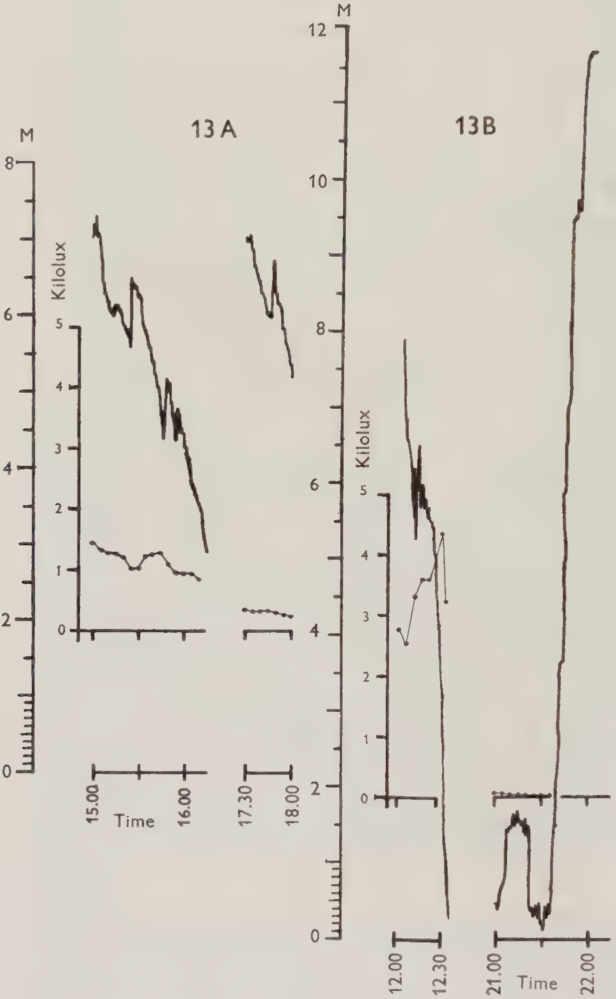
Text-fig. 12 shows some of the results of this series of observations depicted in a graphical form. The speed of swimming of each animal has been calculated for successive 5 min periods and expressed as cm/5 min. These values, termed positive if the animal was moving upwards, negative if downwards, are plotted with a scale representing time of day running horizontally. Those values pertaining to the same animal are joined by a distinctive line. Examination of this figure shows clearly the absence of upward migrations early in the day and the absence of marked downward ones late in the day.

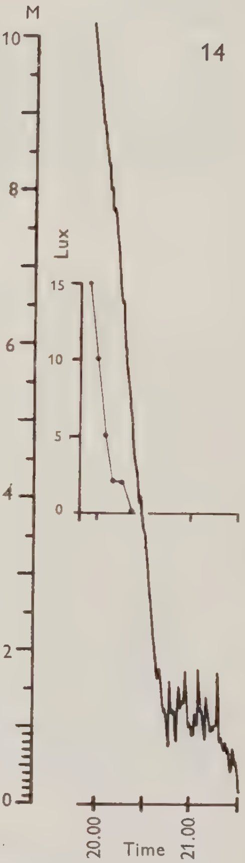
It is clear that the typical daytime behaviour, under the light intensities experienced in the wheel, is a downward swimming, and such is shown in the early parts of a number of records, for example as shown in Text-fig. 13. (The upward swimming of the 'surface' *Calanus* mentioned elsewhere is of



Text-fig. 12. Graph showing the variations in the speeds of swimming, either upwards or downwards, of three specimens of *Calanus finmarchicus* (♂, ♀, and stage V) at successive 5 min intervals during 1¼ h periods. The order of making the recordings is shown by the figures in circles; ♂ ⑦ and ♀ ④ and ⑤ being taken on the day following the remainder of the records.

course an exception to this.) Later in the day this downward movement generally becomes slower and changes into an indeterminate one comprising sporadic bursts of both upward and downward swimming, as is shown in Text-figs. 14–16. Finally, later still, this indeterminate movement changes into a rapid and constant upward migration (records 38, 11, and 24 in

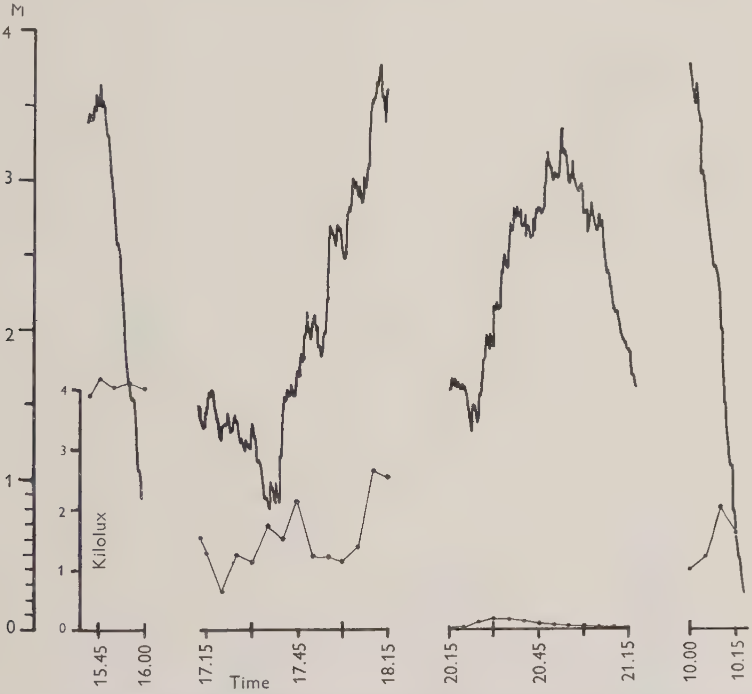




Text-fig. 13. Continuous tracing from kymograph record no. 38 showing the behaviour of a *Calanus* ♂ (caught at the surface) experimented with (A) on 4 May 1951 and (B) on the following day; each also shows graphs of light-intensity.

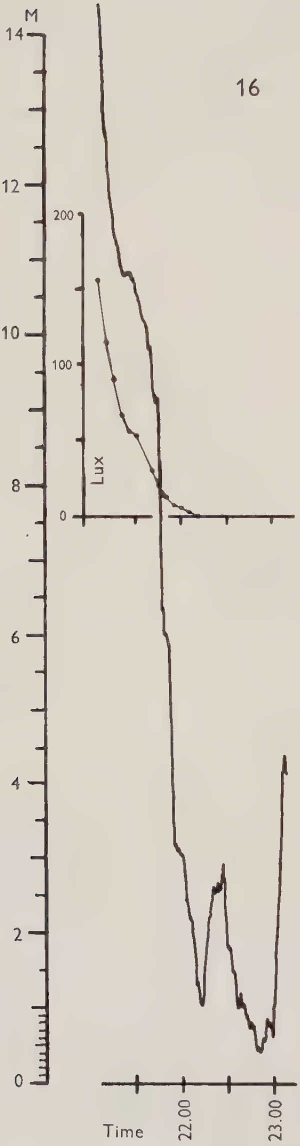
Text-fig. 14. Continuous tracing from kymograph record no. 32 showing the behaviour of *Calanus* on 14 September 1950 together with light-intensity graph.

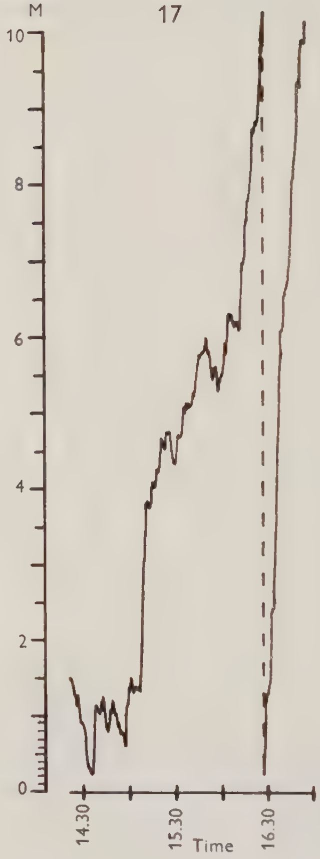
Text-figs. 13, 17 and 18). This cycle is not always followed, however, as in no. 29 (Text-fig. 19), when an animal starts what might have been interpreted as the evening rise, only later (22.10 h) to take to downward swimming again. The latter here can hardly be considered a midnight sinking for it is a definite swimming downward, not an irregular downward wandering. Records nos. 25 and 26 also show resumption of a rapid downward movement but, as would be expected, on the following day.



Text-fig. 15. Continuous tracing from kymograph record no. 36 showing the behaviour of *Calanus* ♂ (caught at the surface) and experimented with on two consecutive days (2 and 3 May 1951), together with light-intensity graphs.

The evening migration in the sea is generally thought to begin about sundown, although of course we know nothing of the possible variation between individuals. In this respect it is interesting to consider the times at which upward swimming began in the various records we have obtained. Clear upward migrations in records nos. 24, 38 and 11 start respectively at 21.15, 21.30 and 16.05 h, while the abortive rise in no. 29 is at 21.35 h. Of these times 16.05 is well before sundown at the time of the experiment; 21.15 is 10 min after; 21.30, 1 h; and 21.35, 1 h 20 min after. In no. 32 (Text-fig. 14) the downward migration changed to an indeterminate swimming at 20.45 h, again 1 h 20 min after sundown. In record no. 36 (Text-fig. 15) a similar change occurred at least at 17.15, although possibly earlier,



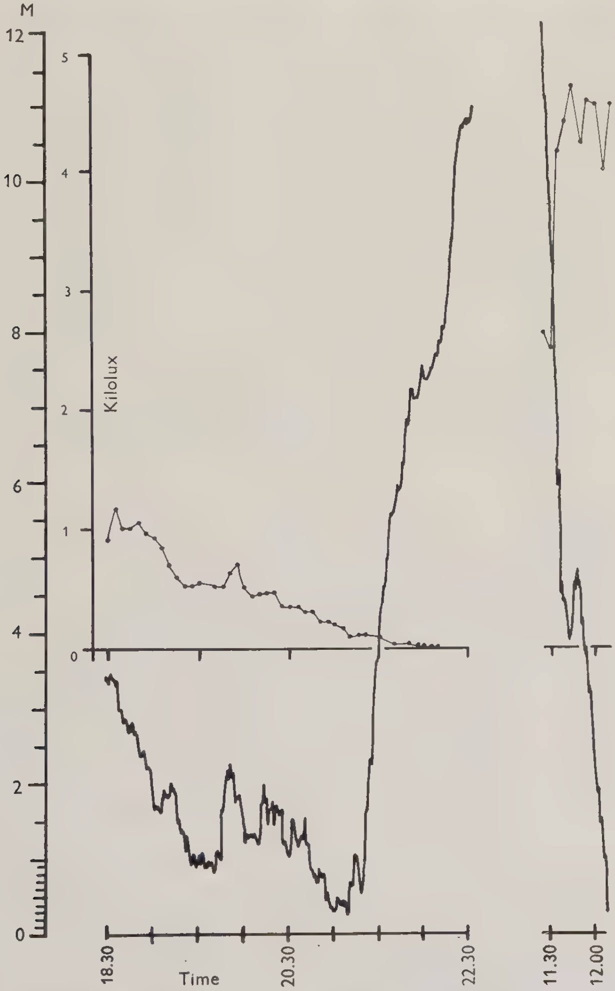


Text-fig. 16. Continuous tracing from kymograph record no. 27 showing the behaviour of *Calanus*, stage V, on 22 July 1950, together with light-intensity record.

Text-fig. 17. Continuous tracing from kymograph record no. 11 showing the behaviour of *Calanus* ♀ on 15 June 1950.

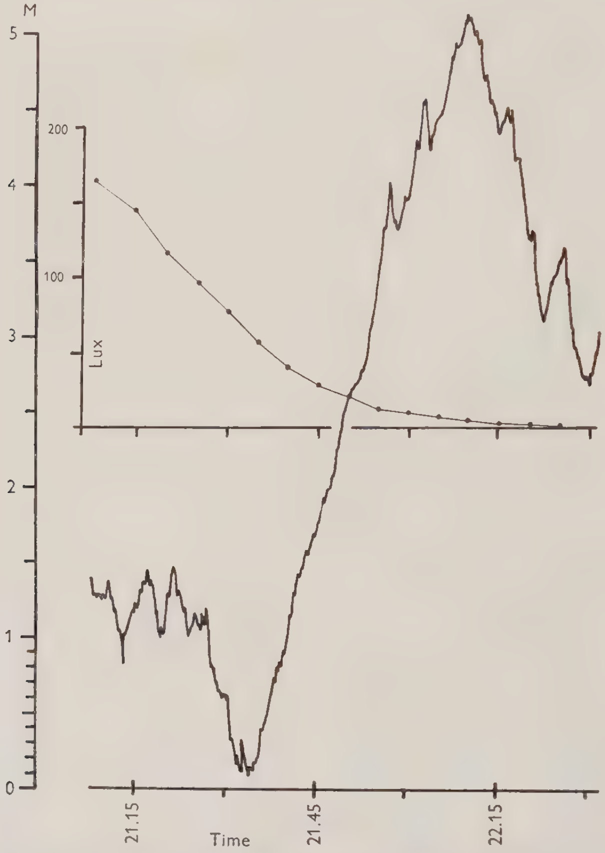
before the second part of the record began; but this particular *Calanus* was one of those found swimming near the surface.

Esterly (1917) gives evidence for the existence of a rhythm of upward migration, persisting even in continued darkness. It must therefore be considered whether those upward migrations obtained appear independent of the light intensity existing at the time or whether migration sets in at a particular intensity rather than at a particular time. In no. 24 (Text-fig. 18), upward movement starts when the light intensity has fallen to 125 lux, in no. 29



Text-fig. 18. Continuous tracings from kymograph records nos. 24 and 25 showing the behaviour of the same *Calanus* stage V on 21 July 1950 and on the following morning, together with light-intensity records.

(Text-fig. 19) when it is at 60 lux and in no. 38 (Text-fig. 13) not until a zero reading was obtained. In no. 32 (Text-fig. 14) also, indeterminate movement set in at a zero intensity but in no. 36 there was no upward movement even at zero lux. It cannot be said how far all the changes of behaviour enumerated in this section may be due to the passage of time, and how far they may be due to the decrease in light intensity necessarily associated with the advancing time of day. It seems possible, however, as upward migrations could not be induced by blacking out during the daytime, that there may be a rhythm in the capacity to respond to a great reduction in light intensity. This would account for the lateness of those migrations that were obtained, as the intensity just below the surface must reach that equivalent to the sundown intensity at 50 or 60 m depth, much later in time.



Text-fig. 19. Continuous tracing from kymograph record no. 29 showing the behaviour of *Calanus* stage V on 25 July 1950, together with light-intensity record.

It is known from other experiments that the behaviour of *Calanus*, in particular, changes with prolonged captivity. This was again found to be so and a greater proportion of animals swimming up was always found amongst samples that had been obtained several days previously. For this reason animals were experimented with as soon as possible after the time of capture.

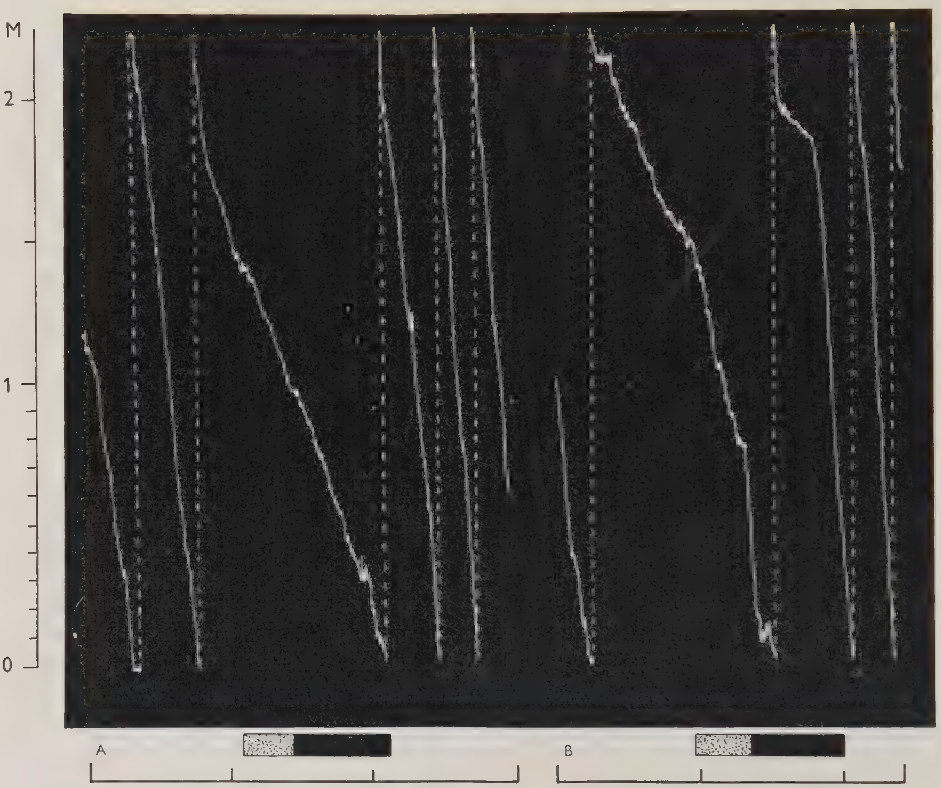
Correlation of behaviour with changes in light intensity

It has for a long time been considered, and indeed from time to time clearly demonstrated, that the vertical migrational behaviour of plankton animals is in part influenced by changes in light intensity. As already explained (p. 415) the wheel apparatus was mounted in a greenhouse whose sides were covered with layers of tissue paper to represent a dome of light, brightest above and falling off at the sides, similar to conditions in the sea; then the actual intensity of the overhead light could be varied from full daylight to complete darkness by drawing over more and more sheets ranging from white, through grey to black. This latter process, however, cannot be made as gradual as the changes in light intensity experienced by the animals in the sea. As also explained, for the majority of records, readings of light intensity were obtained by a photo-cell and sensitive galvanometer (see p. 415). We were thus able to obtain many records of the behaviour of individual animals under known conditions of illumination.

First we will consider seven records for which we have no light intensity readings but during which the light was varied from daylight to complete darkness as explained above. Record no. 1, illustrated in Text-fig. 3 (p. 418), has already been discussed as an example of the method on p. 419, and here we noted that the gradual blacking out to complete darkness and return to daylight had no apparent effect on the behaviour of the *Calanus* then used.

Record no. 9 (not reproduced here) showed, in the first part, a large *Meganyctiphanes norvegica*, observed in the wheel from 11.38 to 12.45 h; it was swimming down rapidly and slightly increasing its speed as the light began to be reduced. As soon as complete darkness was reached the descent was checked and then, after some up-and-down movement, continued but at a slower rate and with a number of pauses. A new part of the record was started at 14.42 with another specimen of *M. norvegica*; one of medium size. This time the record started in complete darkness and the animal began by making almost as rapid a descent as did the other specimen in full daylight, but after about 15 min its behaviour changed, its descent being broken by many short up-and-down movements. When full light was restored it descended again but less rapidly than in the dark, and it was seen to pause for a time at regular intervals which, from their distance apart, must have corresponded to the position of the doors at the junctions between the sections of the wheel; although transparent they may produce a slight shadow.

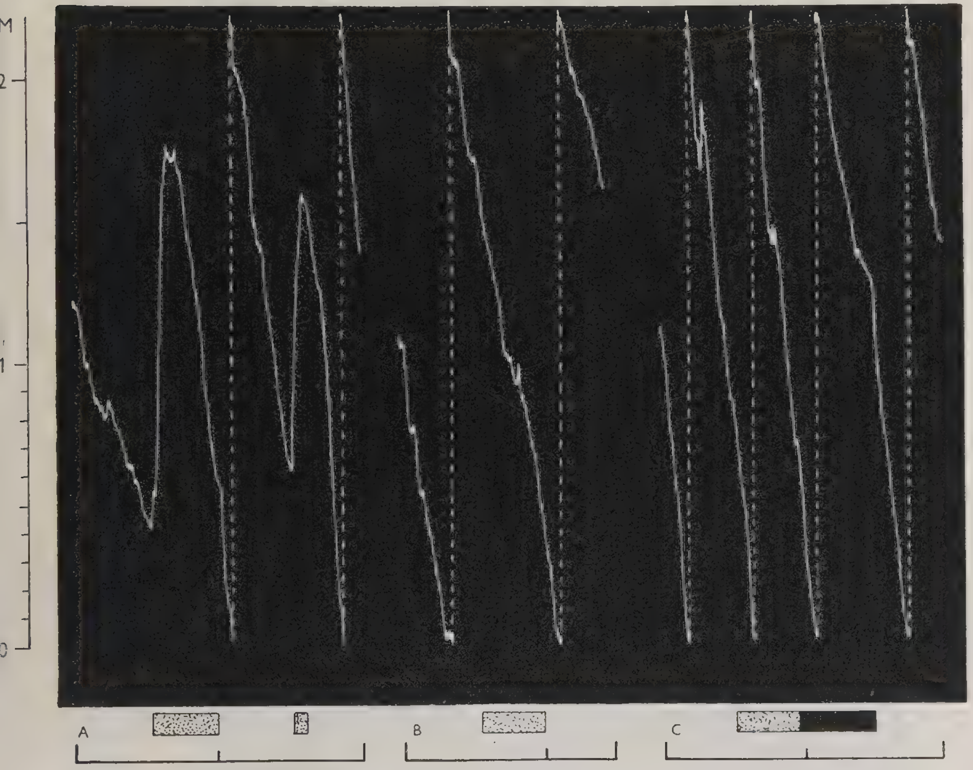
In no. 31 (also not reproduced), *Calanus finmarchicus* began by descending, then went up for a short distance and then down more steeply. Descent now continued till the end of the record at 16.00 h, but during the period in which the light was reduced—either to a partial blackout or to complete darkness—the speed of descent was also reduced; on full daylight being restored again the speed of descent increased once more.



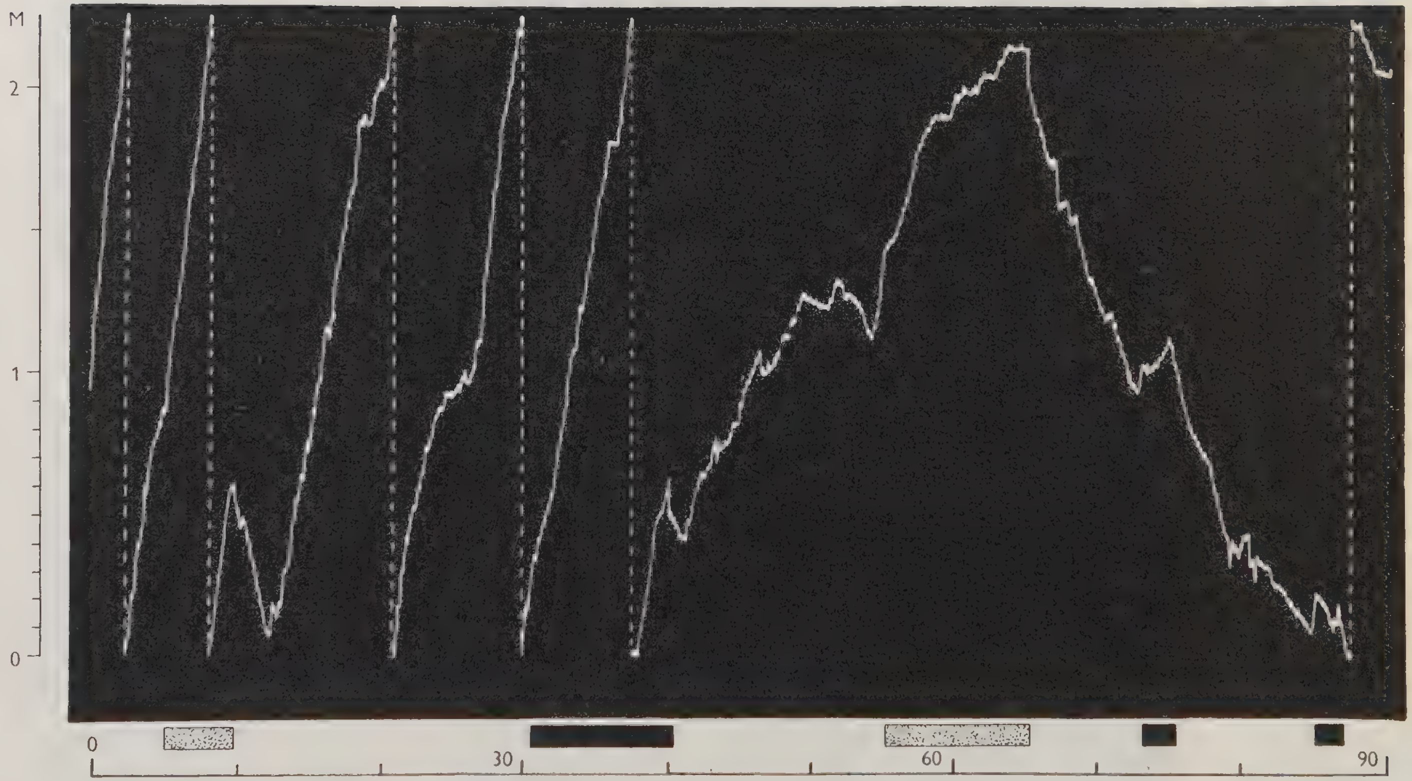
Text-fig. 20. Reproduction of kymograph tracing from record no. 42. A, behaviour of a specimen of *Cypris* sp. observed from 16.35 to 17.05 h on 15 May 1951. B, the same specimen from 10.20 to 10.45 h on 16 May 1951. The stippled and blackened rectangles indicate respectively periods of partial shading and complete darkness. The time scale is divided into 10 min periods. See text below.

Text-fig. 20 shows record no. 42 which follows the descent of a freshwater *Cypris* (species undetermined). Here the recording paper is moving at 5 mm per min. The first part, made from 16.35 to 17.05 h on 15 May 1951, shows a similar reduction in the speed of downward movement during the partial and complete blackout, as observed in the previous record for *Calanus*; the second part, however, 10.20–10.45 h on the following day, shows a reduction of speed before the blackout and an increase in speed again before the daylight was restored.

No. 43, made also on 16 May 1951, shows in Text-fig. 21 the varying behaviour of freshwater *Cyclops*. The first part is with a male from 12.25 to 12.45 h, and each time a partial blackout is introduced the downward swimming is at once changed to an upward movement; on the first occasion the animal swims up 1·35 m and then begins to descend again, although the partial blackout is still maintained, while the next time the full light is restored before the animal has climbed more than 1 m and it descends again as soon as the light increases. The second part of the record is made from 15.10 to 15.25 h with a female *Cyclops*, but here the partial blackout appears to have no effect upon its downward movement. In the third part, 15.25–15.45, the same male as



Text-fig. 21. Kymograph tracing from record no. 43. A, behaviour of *Cyclops* sp. ♂ from 12.25 to 12.45 h on 16 May 1951; B, *Cyclops* sp. ♀ from 15.10 to 15.25 h on 16 May 1951; C, same as in A from 15.25 to 15.45 h also 16 May 1951. The stippled and blackened rectangles indicate respectively periods of partial shading and complete darkness. The time scale is divided into 10 min periods. See adjacent text.



Text-fig. 22. Kymograph tracing from record no. 44 (16 May 1951). Behaviour of *Daphnia* sp. from 17.10 to 18.40 h. Stippled and blackened rectangles indicate respectively periods of partial shading and complete darkness. The time scale is divided into 10 min periods. See text opposite.

was used in the first part is again subjected to a partial blackout which is then followed by complete darkness; this time its behaviour appears quite unaffected.

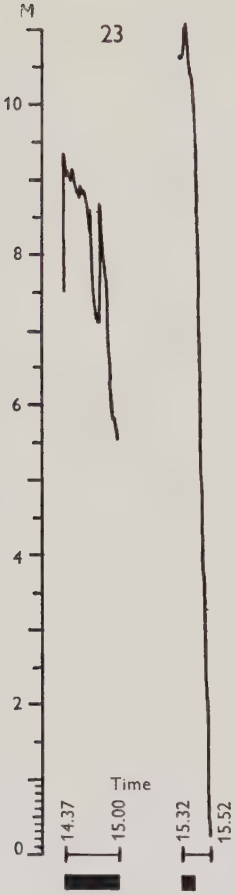
No. 44 shows the behaviour of *Daphnia* species from 17.10 to 18.40 h with the light reduced or completely cut off for short periods as indicated below the graph in Text-fig. 22. The changes in light intensity have only a limited effect upon its general movement, which is upward for just over 1 h and then downward. After the first partial blackout there is a short period of downward swimming immediately the full light is restored, similarly for a shorter period after the first full blackout. Just before the second partial blackout there is an indication that the animal was beginning to descend, but it at once swims quickly upward for a short time as soon as the light is reduced, then after a slackening in climbing it begins its main descent when the light increases again. Its descent, however, is checked and changed to an upward movement at a second complete blackout, and is checked again but not so effectively at the third and last short period of darkness. A further experiment with *Daphnia* is shown in Text-fig. 28 and discussed on p. 443.

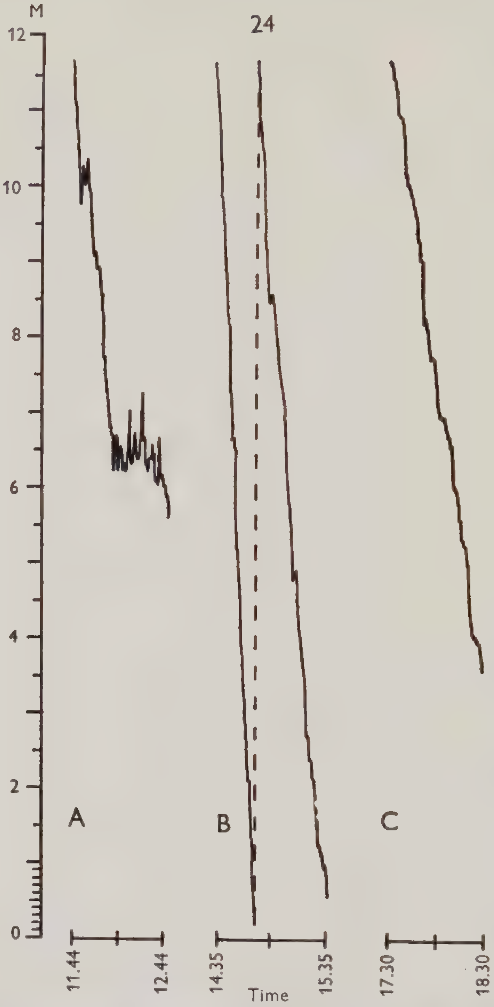
Record 47 (Text-fig. 23) shows the movement of a *Calanus* ♀ for 23 min in complete darkness, 14.37 to 15.00 on 13 July 1951, when it rises and falls, rises and falls; then in a second 20 min period when full light is restored after 10 min the same animal makes a continuous and rapid descent of some 11 m.

Before passing to the records accompanied by actual light-intensity measurements reference should be made to record no. 17 where the behaviour of a *Calanus* female is recorded on 22 June 1950 in three parts: 11.44–12.44 h with 'heavy cloud and no sun', 14.35–15.35 h with 'some cloud and strong sun' and 17.30–18.30 h 'some cloud but little sun'. It is shown in Text-fig. 24, having been retraced on a reduced scale to give a more continuous graph. Here the speed of descent appears to show some correlation with light. In the first hour, about midday, during which there is heavy cloud and no sun, it descends only 6 m; in the last hour, towards evening, but when there is a little sun, it descends 8 m; whereas in the full afternoon with strong sun it descends nearly 23 m.

The remaining figures in this section have all been retraced from those kymograph records accompanied by light-intensity measurements; the latter are shown with them. Record no. 26 in Text-fig. 25, from 15.00 to 18.30 on 22 July, shows *Calanus* stage V descending and subjected to a complete blackout; its speed of descent is appreciably reduced during darkness and greatly increased again as soon as light is restored. Text-figs. no. 26 and 6 (pp. 443 and 423) are parts of the same record, no. 28, taken on 25 July 1950; the first part from 17.20 to 18.20 h recording *Calanus* stage V and the second part 18.30 to 21.00 h recording *Labidocera wollastoni* ♂. In the second part the light intensity is a good deal lower than in the first part and is shown on a different scale. In each case as the light declines so the downward movement of the animal is checked.

Text-fig. 27 shows the behaviour of a *Calanus* stage V in record no. 37, from 14.00 to 16.30 and 17.00 to 17.30 h on 3 May 1951, and from 10.00 to 10.50 h on the following day. In the first period it begins by climbing some 9 m and then makes an equally rapid descent of 18 m when there is a check for half an hour followed by a further rapid descent; its movement shows no correlation at all with the light intensity which falls from 5·6 to 4 kilolux with



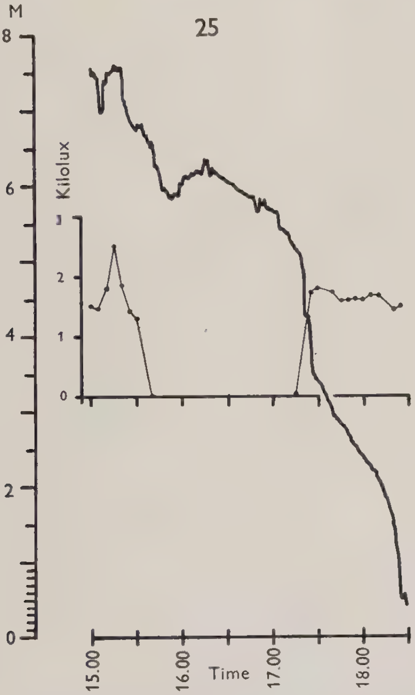


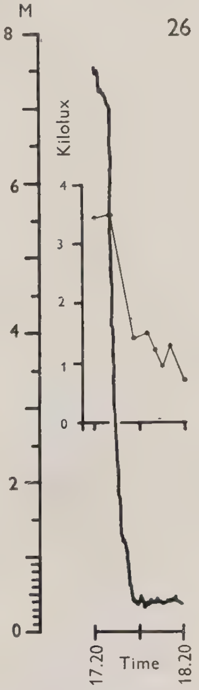
Text-fig. 23. Continuous tracing from kymograph record no. 47 (13 July 1951) showing behaviour of *Calanus* ♀ with artificial blackout.

Text-fig. 24. Continuous tracing from kymograph record no. 17 (22 June 1950) showing the behaviour of *Calanus* ♀ under different conditions of cloud and sunlight (see text, p. 441): A, heavy cloud, no sun; B, some cloud, strong sun; C, some cloud, little sun.

fluctuations due to passing clouds. It is likewise impossible to see any obvious correlation in the other two periods.

Text-fig. 28 shows record no. 39 made with *Daphnia* sp. on 7 May 1951. In the first period of half an hour the light is raised from 4 to 8 kilolux and then down to darkness; in the second period of half an hour it is raised from darkness to 6 kilolux and back to darkness again, but on neither occasion does the downward movement of the animal appear to be affected. In the third period, however, from 14.45 to 16.10, the behaviour of the same animal is markedly different; as soon as the light falls from 6 kilolux to zero the *Daphnia* climbs for some 6 m and then, when the light is brought back to 6 kilolux, it descends as steeply for nearly 15 m only to start climbing again as the light is again dimmed; finally it falls yet again as the light is once more increased.

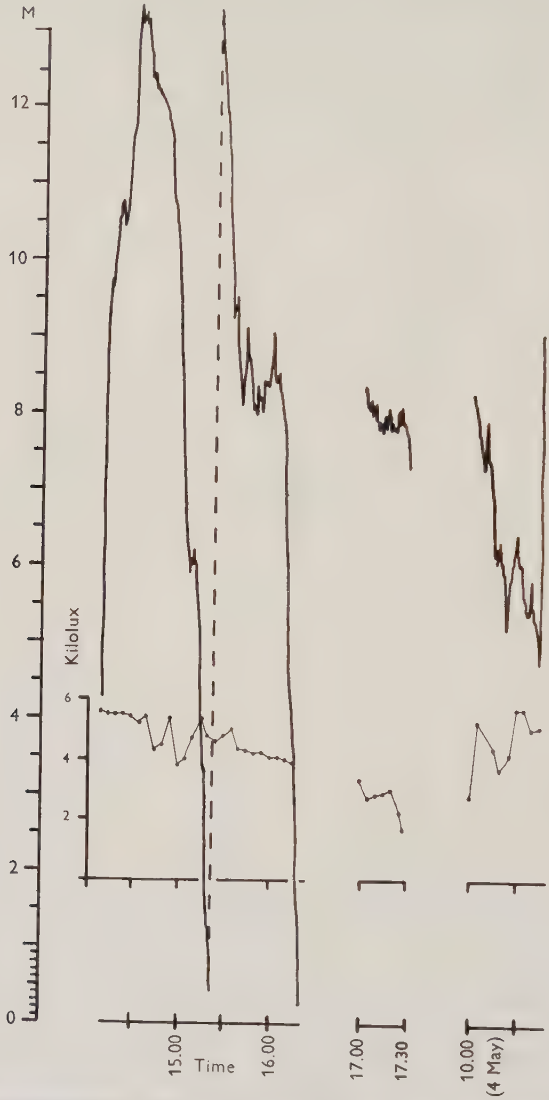




Text-fig. 25. Continuous tracing from kymograph record no. 26 (22 July 1950) showing the behaviour of *Calanus* stage V with artificially produced blackout as shown in light-intensity graph.

Text-fig. 26. Continuous tracing from part of kymograph record no. 28 (25 July 1950) showing the behaviour of *Calanus* stage V with decreasing light intensity as shown in accompanying graph.

Record no. 41, illustrated in Text-fig. 29, is particularly interesting because it is made with one of the spring brood of *Calanus*, a female taken at the surface in bright light at Millport on 12 May 1951. The record is from 12.10 to 12.50 with a 5 min break at 12.25. The animal starts by climbing steeply while the light is increased from 5·6 to 7·5 kilolux and continues to



Text-fig. 27. Continuous tracing from kymograph record no. 37 (3 and 4 May 1951) of *Calanus* stage V showing behaviour which appears to have no correlation with accompanying light-intensity graph.

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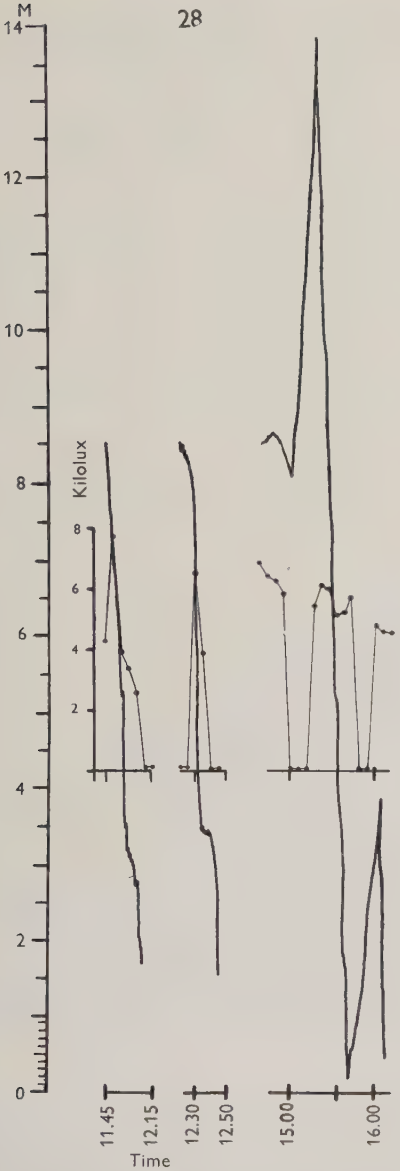

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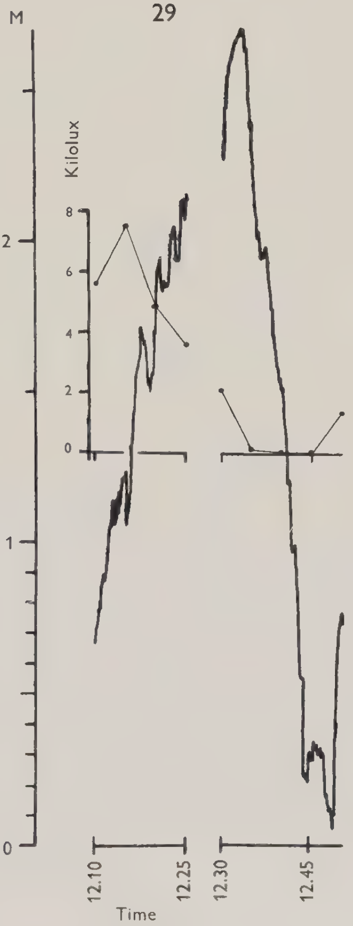

Text-fig. 28. Continuous tracing from kymograph record no. 39 (7 May 1951) showing the behaviour of *Daphnia* sp. under artificial control of light as shown in light-intensity graph. See text, p. 443.

Text-fig. 29. Continuous tracing from kymograph record no. 41 (12 May 1951) showing the behaviour of *Calanus* ♀ caught at the surface and placed under artificially controlled light as shown in accompanying graph. See text, p. 444.

climb as the light then falls again; the light is now reduced gradually and as it falls below 1 kilolux the animal suddenly begins to swim rapidly down and continues to do so while the light remains at zero. When the light starts to increase again the descent of the animal is checked for a few minutes before it goes down a little further, and then, as soon as the light intensity reaches 1 kilolux, up it swims again with great speed.

In addition to the records considered here we should also refer back to some of those already discussed in the previous section dealing with changes of behaviour with time. Records nos. 24, 29 and 38 show an upward evening migration starting at the very different light intensities of 125, 60 and 0 lux respectively (Text-figs. 18, 19 and 13). No. 32 shows in Text-fig. 14 a downward migration changing to an indeterminate up-and-down movement when the light falls to complete darkness. No. 36 (Text-fig. 15) shows some upward swimming of a 'surface' *Calanus* at much higher light intensities.

It is clear that on some occasions a change in light intensity will have an immediate effect upon the behaviour of an animal, but at other times, even with the same individual, it appears to have little or no influence at all.

Discussion

While stressing that this account, apart from the description of this new method of experimentation, must be largely preliminary, it may nevertheless be worth considering to what extent the results so far obtained are consonant with current theories of vertical migration.

The experiments clearly show that all the migrations reported on the evidence of net hauls are well within the capabilities of the animals concerned. The table of speeds shows that they can swim both quickly enough and for sufficiently long periods to cover the distances involved in even less time than has previously been considered necessary. It is possible of course that the stimulus of confinement in the wheel may cause them to swim faster than they would in nature, but the capacity to maintain such speeds for long periods is clearly demonstrated. The tendency amongst the forms investigated, to swim downwards as distinct from passively sinking, is especially interesting in verifying that downward migrations can take place as quickly as, or even faster than, upward ones.

The influence of fluctuations in light intensity upon the movements of the animals tested, while giving some confusing results, is further evidence as to the importance of this factor. Except for *Centropages* (and specimens of *Calanus finmarchicus* found at the surface in daylight during the spring), all the animals tested exhibited a negative reaction towards strong light and swam downwards and away from it at some of the highest speeds. A gradual reduction in intensity is usually accompanied by a diminution in this reaction and at low values in the evening it may be replaced by a migration upwards and towards the light. The artificial removal of all light during the daytime does not on most occasions evoke an upward movement, and so it is clear that there is not a negative geotaxis which comes into play in the absence of the inhibiting effect of strong light; there is clearly demonstrated, again on most but not on all occasions, a definite positive migration towards the source of low light intensities. If these latter reactions should occur in the sea then most animals, if they were reacting in the same way, would automatically accumulate in regions of a certain light intensity (which may conveniently be termed the optimum) and move up to the surface as the level of this intensity rises with the setting sun; this indeed is as postulated in the hypotheses of Michael (1911) and Russell (1926). The movement towards the optimum conditions is shown generally not to be a random or 'indirect' one but, in most of the larger forms, to consist of sustained directional swimming either up or down; this appears to be replaced by a 'hop-and-sink' type of movement when the region of optimum conditions has been reached.

This simple picture of daily change is not always true, however, and sometimes (as with the upward swimming of some of the surface members of the spring brood of *Calanus*) it may be completely reversed. *Centropages* exhibited this reverse type of behaviour whenever we observed it, and the effect of this in the sea would be to keep individuals near the surface during the hours of daylight. Even apart from these regular exceptions, however, the variation met with among the different animals experimented with shows that their migrations cannot be governed by changes in light intensity alone. In record no. 43 (p. 439), for example, we have seen that a partial blackout at midday caused a *Cyclops* male to swim upwards at once, whereas in the afternoon of the same day both a partial and a complete blackout had no effect whatever on the same individual. Similarly, with *Daphnia* in record no. 39 (Text-fig. 28, p. 445) marked alterations in light intensity *in the morning* on this occasion had no effect on its behaviour, whereas *in the afternoon* a complete blackout caused the same individual to swim rapidly upwards on two occasions. These results clearly show that a great many more observations are required before we can determine the relative importance of light and other factors in the regulation of vertical migrational behaviour.

SUMMARY

An apparatus is described in which it is possible to observe and make continuous records of the vertical migrations of plankton animals, if necessary through hundreds of metres of water.

Records obtained with this apparatus are described and discussed.

It is considered that vertical migration in the sea is predominantly a direct up-and-down swimming with only a few smaller forms moving in an indirect way. Downward migration is shown to be an active swimming and not a passive sinking.

The speeds of vertical swimming of various animals are measured and listed.

Much variation and 'individuality' is shown amongst the animals studied but a pattern of behaviour consisting of a rapid movement downwards and away from the bright light during the day, followed by a diminution of this as the light intensity falls and an eventual movement upwards under low light intensities, appears to be usual. Upward migration cannot generally be induced by blacking out during the daytime and is assumed to be a positive migration towards low light intensities rather than a negative geotaxis.

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ADDENDUM

Since going to press a very interesting paper by Professor J. E. Harris has appeared, on the 'Physical factors involved in the vertical migration of plankton' (*Quart. J. micr. Sci.*, Vol. 94, pp. 537–50, Dec. 1953). We cannot agree that all his conclusions, based upon the study of *Daphnia magna*, for example those concerning a negative geotaxis, can apply to all plankton animals which migrate vertically. It may however be significant that the animals which we found at times swimming horizontally were mainly those having, like his *Daphnia*, compound eyes: euphausians and decapod larvae (see p. 424).

J. Mar. biol. Ass. U.K. (1954) 33, 449–455
Printed in Great Britain

THE VAPOUR PRESSURE AND OSMOTIC EQUIVALENCE OF SEA WATER

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Sea water is a complex solution in which the principal ions are sodium, potassium, calcium, magnesium, chloride and sulphate. The vapour pressure (V.P.) of such a solution can be calculated approximately by making the assumption that each salt contributes to the vapour pressure lowering in amount proportional to its concentration, but such a calculation would ignore the interactions between the various ions. The theory of these interactions has been worked out only for very dilute solutions and it is, therefore, better to rely on direct experimental determinations. Measurements have now been made by the isopiestic vapour-pressure method (Robinson & Sinclair, 1934), in which samples of sea water are equilibrated with sodium chloride solutions until they have the same vapour pressure. The results are expressed in terms of chlorinities of sea water and molalities (moles per kilogram of H_2O) of sodium chloride solution which have the same vapour pressure. It is hoped that the results will be of use to physiologists who have occasion to make up salt solutions equivalent to sea water.

EXPERIMENTAL

Three samples of sea water were used:

(1) Eau de mer normale, P 17, 31 October 1948, ‰ Cl = 19·386; found by gravimetric analysis, 19·408 ‰* (i.e. by precipitation as silver halide, calculated as silver chloride).

(2) An artificial sea water made up as follows:

	g/kg solution		g/kg solution
Sodium chloride	28·85	Calcium chloride	1·244
Potassium chloride	0·811	Magnesium sulphate	3·649
Magnesium chloride	2·633		

The composition is quoted in terms of anhydrous salt. Found by titration against Eau de mer normale: ‰ Cl = 20·58; by gravimetric analysis: 20·62.

(3) Sea water taken from the Straits of Singapore. Found by titration against Eau de mer normale: ‰ Cl = 17·27; by gravimetric analysis: 17·35.

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* This figure includes the weight of bromine in excess of the equivalent of chlorine. If allowance is made for this, and the new figure divided by 1·00045 to allow for change in atomic weights since 1937, the gravimetric chlorinity becomes 19·390 ‰. Similarly the gravimetric chlorinities of samples 2 and 3 become 20·60 ‰ and 17·33 ‰, agreeing with the titration chlorinities even better than the author claims.—(Ed.)

The densities of these three solutions were found to be $d_4^{25} = 1{\cdot}0233_4$, $1{\cdot}0249_8$ and $1{\cdot}0206_2$ respectively. The results of this investigation are all expressed in terms of chlorinities as found by titration.

In the isopiestic method samples of sea water are weighed in two platinum dishes, and samples of a NaCl solution of known composition are weighed out into two other platinum dishes. The four dishes are then placed on a copper block in a desiccator which is evacuated and rocked gently in a thermostat at 25° C for 2 days. During this interval water distils from one solution to another until equilibrium is reached when the concentrations of all four solutions are such that the vapour pressures of all four are equal. The dishes are then weighed again and, from the loss or gain in weight, the final concentrations of the solutions are calculated. These solutions of equal vapour pressure are said to be isopiestic and the ratio, R, of the concentration of the sodium chloride solution to that of the sea water is called the *isopiestic ratio*. If the vapour pressures of solutions of sodium chloride are known as a function of their concentration, and tables of such vapour pressures have been published (Robinson, 1945; Stokes & Levien, 1946), then the vapour pressure of the sample of sea water can be calculated for a particular concentration. Thus, in one experiment, a sea-water solution of 20·02‰ chlorinity was found to have the same V.P. as 0·5889 M-NaCl solution; the relative molal V.P. lowering of NaCl, $(p^0-p)/mp^0$, where p^0 is the V.P. of pure water and p is the V.P. of NaCl solution of molality m, is 0·03290 at 0·5 M and 0·03292 at 0·6 M. It may be taken as 0·03292 at 0·5889 M and the relative vapour pressure lowering $(p^0-p)/p^0$ or $\Delta p/p^0$, as $0{\cdot}03292 \times 0{\cdot}5889 = 0{\cdot}01939$. If the V.P. is required we put $p^\circ = 23{\cdot}756$ mm at 25° so that $(p^0-p) = 0{\cdot}461$ mm and $p = 23{\cdot}295$ mm. This is also the V.P. of 20·02‰ Cl sea water.

The experiment is repeated at a number of different concentrations to investigate the change in V.P. over a range of concentrations. Fourteen measurements were made using the three sea-water samples and the results are given in Table I. Over the range 9–22‰ Cl, the ratio of NaCl molality to sea-water chlorinity can be expressed as

$$R = 0{\cdot}02782 + 0{\cdot}000079\ (‰\ \text{Cl}),$$

a formula which expresses the results in Table I with an average deviation of 0·18%.

Discussion

The above equation can be used to calculate values of R at round values of the chlorinity between 10 and 22‰. These are recorded in Table II. The third column of the table gives the molality of NaCl solution of the same V.P. as the sea water whose chlorinity is given in the first column. A very careful study has been made (Robinson, 1945) of the ratio of the molalities of NaCl and KCl solutions which are isopiestic (i.e. have the same V.P.), and it is therefore possible to give in the fourth column the molalities of KCl solutions

TABLE I. MOLALITIES OF SODIUM CHLORIDE SOLUTIONS AND CHLORINITIES OF SEA WATER OF THE SAME VAPOUR PRESSURE

			R	
Sample	M-NaCl	‰ Cl	Observed	Calculated
1	0·4296	14·79	0·02905	*0·02899*
	0·5454	18·62	0·02929	*0·02929*
	0·5847	19·90	0·02938	*0·02939*
	0·6185	21·01	0·02944	*0·02948*
2	0·2700	9·44	0·02860	*0·02857*
	0·3774	13·08	0·02885	*0·02885*
	0·4220	14·60	0·02890	*0·02897*
	0·4350	15·04	0·02892	*0·02900*
	0·4737	16·35	0·02897	*0·02911*
	0·5492	18·72	0·02934	*0·02930*
	0·5889	20·02	0·02942	*0·02940*
	0·6171	20·96	0·02944	*0·02948*
3	0·4628	15·87	0·02916	*0·02907*
	0·5753	19·52	0·02947	*0·02936*

R = M-NaCl/‰ Cl

TABLE II. VAPOUR PRESSURE AND OSMOTIC EQUIVALENCE OF SEA WATER AT 25° C

‰ Cl	R	NaCl	KCl	$CaCl_2$	$MgCl_2$	$MgSO_4$	Na_2SO_4	Sucrose	Urea	V.P. lowering	Osmotic pressure (atm.)
10	0·02861	0·2861	0·2908	0·2039	0·2005	0·5056	0·2374	0·5065	0·5400	0·00946	12·87
11	0·02869	0·3156	0·3211	0·2240	0·2199	0·5597	0·2643	0·5560	0·5965	0·01042	14·19
12	0·02877	0·3452	0·3516	0·2441	0·2393	0·6138	0·2918	0·6053	0·6534	0·01139	15·51
13	0·02885	0·3751	0·3825	0·2642	0·2588	0·6675	0·3196	0·6546	0·7112	0·01237	16·85
14	0·02893	0·4050	0·4134	0·2841	0·2780	0·7206	0·3477	0·7040	0·7695	0·01334	18·19
15	0·02901	0·4352	0·4447	0·3043	0·2975	0·7738	0·3762	0·7534	0·8285	0·01433	19·55
16	0·02908	0·4653	0·4760	0·3243	0·3165	0·8264	0·4051	0·8025	0·8880	0·01532	20·91
17	0·02916	0·4957	0·5077	0·3445	0·3356	0·8786	0·4347	0·8516	0·9482	0·01631	22·28
18	0·02924	0·5263	0·5397	0·3645	0·3546	0·9300	0·4648	0·9008	1·010	0·01732	23·66
19	0·02932	0·5571	0·5719	0·3845	0·3738	0·9803	0·4954	0·9497	1·071	0·01833	25·06
20	0·02940	0·5880	0·6043	0·4044	0·3929	1·028	0·5264	0·9982	1·133	0·01936	26·47
21	0·02948	0·6191	0·6370	0·4243	0·4122	1·076	0·5578	1·047	1·197	0·02039	27·89
22	0·02956	0·6503	0·6698	0·4440	0·4313	1·123	0·5896	1·095	1·260	0·02142	29·33

The column headed V.P. lowering gives the relative pressure lowering $\Delta p/p^0 = (p^0 - p)/p^0$, where p is the vapour pressure of the sea water and p^0 is the vapour pressure of pure water. $p^0 = 23{\cdot}756$ mm at 25° C.

isopiestic with sea water. Similar comparisons of $CaCl_2$ with NaCl (Stokes, 1945*a*), $MgCl_2$ with KCl (Robinson & Stokes, 1940; Stokes, 1945*b*), $MgSO_4$ with KCl (Robinson & Jones, 1936), Na_2SO_4 with KCl (Robinson, Wilson & Stokes, 1941), sucrose with KCl (Robinson & Sinclair, 1934; Scatchard, Hamer & Wood, 1938; Robinson, Smith & Smith, 1942) and urea with NaCl (Scatchard *et al.*, 1938) have been made, enabling us to give in the next six columns of Table II, molalities of various solutions of the same V.P. as sea water. The solutions whose concentrations are given in any one row of Table II have the same V.P. and the same (thermodynamic) water activity; it is not claimed that any of them can be mixed without change in V.P. We know little about the V.P. of mixed salt solutions but what information is available suggests that whilst solutions of NaCl, KCl, and perhaps $CaCl_2$ and $MgCl_2$, can be mixed without significant change in V.P., the admixture of any one of these with $MgSO_4$ may lead to a marked change in V.P.

In the last column but one of Table II are given the V.P. lowerings corresponding to each chlorinity. These can be expressed by the formula

$$(p^0 - p)/p^0 = 0{\cdot}0009206\ (\text{‰ Cl}) + 0{\cdot}00000236\ (\text{‰ Cl})^2,$$

where (‰ Cl) is the chlorinity given in the first column of Table II. The V.P. lowering is therefore not linear in the chlorinity as would appear from the equation of Witting (1908):

$$p/p^0 = 1 - 0{\cdot}000969\ (\text{‰ Cl}),$$

an equation which gives a good representation of the vapour-pressure lowering of sea water only in the vicinity of 20‰ chlorinity. Thus for standard sea water of 19·386‰ Cl, our formula gives $\Delta p/p^0 = 0{\cdot}01874$, compared with 0·01879 by Witting's formula.

The osmotic pressure, Π, of these solutions can be calculated by the formula

$$\Pi = -(RT/V_1)\ln a_W,$$

where V_1 is the partial molal volume of water in the solutions and a_W is the water activity or the relative V.P., p/p^0. It can be assumed without significant error that V_1 can be equated to the value in pure water; that is to say, it is put equal to the molar volume of pure water. Moreover, the osmotic coefficient, ϕ, of the solution, defined by

$$\phi = -(55{\cdot}51/2m)\ln a_W$$

enables us to make the transformation to

$$\Pi = (2mRT\phi)/(55{\cdot}51 V_1).$$

(The osmotic coefficients of these salt solutions have been tabulated and are easier to use in computations than the quantity $\log a_W$; the factor 2 in the above equation is valid for salts dissociating into two ions such as NaCl; for salts like $CaCl_2$ the factor is 3.)

Substituting numerical values at 25°, this equation becomes

$$\Pi = 48{\cdot}8m\phi.$$

Substituting values of ϕ corresponding to the molalities of NaCl in the third column of Table II and using the tables of osmotic coefficients already evaluated (Robinson, 1945; Stokes & Levien, 1946), the osmotic pressures given in the last column of Table II are calculated. They refer to a temperature of 25° C; at another temperature, t° C, the osmotic pressure can be calculated *approximately* by multiplying by the factor $[1 + (t - 25)/298]$.

All these experiments refer to 25° C; none has been done at other temperatures and we can only estimate from other work what the temperature effect is likely to be. One way in which an estimate of the temperature effect can be made is as follows. Thompson (1932) has given a formula for the depression of the freezing-point of sea water:

$$\Delta T = -0{\cdot}0966\,(\text{‰ Cl}) - 0{\cdot}0000052\,(\text{‰ Cl})^3,$$

from which the freezing-point at various chlorinities has been calculated and recorded in Table III. Scatchard & Prentiss (1933) have measured very accurately the freezing-point of NaCl solutions, and from their tables we can find by interpolation the molalities of NaCl solutions which freeze at the same temperature as these sea-water solutions. Solutions of the same freezing-point must have the same v.p. For each of the seven selected chlorinities these NaCl molalities are also given in the table as well as the corresponding NaCl molality at 25° C. It will be seen that the effect of a 26–27° C temperature difference corresponds to only a small change in the NaCl molality, a change of between 0·4 and 0·8% over a chlorinity range of 10–22‰.

TABLE III

‰ Cl	10	12	14	16	18	20	22
Freezing-point depression	0·971	1·168	1·366	1·567	1·769	1·974	2·180
M-NaCl at freezing-point	0·2851	0·3439	0·4028	0·4627	0·5230	0·5839	0·6450
M-NaCl at 25°	0·2861	0·3452	0·4050	0·4653	0·5263	0·5880	0·6503

Finally we may consider the accuracy which can be attained by calculating the v.p. lowering as the summation of the values for the component salts. We can try the assumption that all the chlorinity can be counted as NaCl and find the corresponding v.p. lowering. For example, the standard sea water of 19·386‰ chlorinity would contain 31·96 g NaCl per kg of solution calculated on this assumption, equivalent to 0·5648 M-NaCl. Such a solution has a v.p. lowering of $\Delta p/p^0 = 0{\cdot}01858$ compared with 0·01873 for this sea water (interpolated from Table II). Similarly, the artificial sea water (sample 2) of 20·58‰ chlorinity is calculated as 0·6008 M-NaCl which has $\Delta p/p^0 = 0{\cdot}01978$ compared with the observed (interpolated) value of 0·01996, a difference corresponding to only 0·004 mm of mercury pressure. Alternatively, we could assume that the contribution of each salt is determined by its relative molal v.p. lowering at the total ionic strength of the sea water. For example, the artificial sea water (sample 2), as made up, had the following composition in moles per kg of H_2O:

$NaCl$	KCl	$MgCl_2$	$CaCl_2$	$MgSO_4$
0·5125	0·0113	0·0287	0·0116	0·0315

By taking account of the valencies of these salts the total ionic strength can be calculated as 0·7707. At this ionic strength the relative molal v.p. lowering of each salt can be interpolated from the tables to which reference has already been made, and $\Delta p/mp^0$ found to be 0·03300, 0·03192, 0·04745, 0·04652, 0·02032 for the salts in the order listed above. Hence $\Delta p/p^0$ for these salts is 0·01691, 0·00036, 0·00136, 0·00054 and 0·00064, and the total is 0·01981. The mixed solution had a chlorinity of 20·58‰, and by interpolation in Table II the relative V.P. lowering is 0·01996. The difference between 0·01981 and 0·01996 corresponds to only 0·003 mm of pressure. In the absence of direct measurements, therefore, the V.P. can be calculated with some confidence either from the V.P. lowering of the component salts or by assuming that sea water is a NaCl solution of equivalent chlorinity. It is worth while reiterating, however, that the $MgSO_4$ in these solutions is present in comparatively small amount, and the simple additivity rule might not apply so well if this salt were present in large quantities.

I wish to thank Dr L. H. N. Cooper for a number of valuable suggestions and Mr R. W. Green and Mrs H. Tong for assistance with the analyses.

SUMMARY

Measurements have been made by the isopiestic method of the vapour pressure at 25° C of sea water of chlorinity between 10 and 22‰. A table is given of the concentrations of solutions of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulphate, sodium sulphate, sucrose and urea of equal vapour pressure to these sea waters. Their osmotic pressures are also tabulated.

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J. Mar. biol. Ass. U.K. (1954) 33, 457–472
Printed in Great Britain

A COMPARATIVE STUDY OF THE BRITISH SPECIES OF *NUCULA* AND *NUCULANA*

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(Plate I and Text-figs. 1–8)

The survey of the sublittoral fauna of the Clyde Sea Area from 1949 onwards has shown that five species of the Protobranchiata are abundant throughout this region on a variety of substrata. Pelseneer (1891, 1899, 1911), Heath (1937), and Yonge (1939) have contributed much to the knowledge of the group as a whole, but little comparative work has been done at species level. Verrill & Bush (1897, 1898) studied the shell characters of the American Atlantic species. Moore (1931 *a*, *b*) worked on the faecal pellets of the British Nuculidae and attempted to distinguish the species by this means, while Winckworth (1930, 1931), mainly in the light of the latter work, attempted to clarify the nomenclature of these species. Winckworth (1932) lists six British species of the family Nuculidae: *Nucula sulcata* Bronn, *N. nucleus* (Linné), *N. hanleyi* Winckworth, *N. turgida* Leckenby & Marshall, *N. moorei* Winckworth and *N. tenuis* (Montagu); and four species of the family Nuculanidae: *Nuculana minuta* (Müller), *Yoldiella lucida* (Lovén), *Y. tomlini* Winckworth and *Phaseolus pusillus* (Jeffreys). All species of *Nucula*, except *N. hanleyi*, were taken from the Clyde Sea Area, although the latter species is included in the Clyde fauna list (Scott Elliot, Laurie & Murdoch, 1901). Only *Nuculana minuta* of the Nuculanidae has been taken on the present survey. *Yoldiella tomlini* is included in the 1901 list but is noted as being 'insufficiently attested'. *Nucula hanleyi* was obtained from the Marine Station, Port Erin, but *Yoldiella* and *Phaseolus* were unobtainable.

Difficulty has been experienced in distinguishing the species of *Nucula*. Recent work (Allen, 1953 *a*) shows no differences between *N. turgida* and *N. moorei*, these are now combined under the name *N. turgida* Leckenby & Marshall. Moore (1931 *a*) has also suggested that *N. hanleyi* and *N. nucleus* do not differ from each other, but the present study does not support this view. The specific differences of the British Nuculidae have been investigated and, though no single character is diagnostic, it has been found that the species can be readily distinguished when several characters are considered. An attempt has been made also to correlate specific differences with mode of life. In addition, information on growth rates and length of life has been determined.

Measurements and observations on *Nuculana* have been included where they complete the ecological picture and where they indicate similarities at the generic level.

I wish to express my thanks to Prof. C. M. Yonge, F.R.S., for his criticism of the manuscript; and to my wife for her help and criticism. Thanks are due to the Director and Staff of the Millport Marine Station for their help, and to the skipper and crew of the R.V. *Calanus* for their assistance in obtaining the animals. I also wish to thank Dr N. S. Jones of the Port Erin Marine Station for his kindness in obtaining the sample of *Nucula hanleyi* without which this work would have been incomplete.

This work was carried out in the first place with financial assistance from the Browne Research Fund of the Royal Society and later by a Research Grant from the Development Commission.

Ecology and Habitat

The animals were taken by means of the Agassiz Trawl and the Naturalist's Dredge. Much of the material was taken in 1949–50 from the samples collected in the course of the Clyde fauna survey. Further large samples were taken in 1951 which included *Nuculana minuta* from the Kilbrennan Sound, *Nucula sulcata* from the Cumbrae Deep, and *N. nucleus* from the Minard Narrows, Loch Fyne.

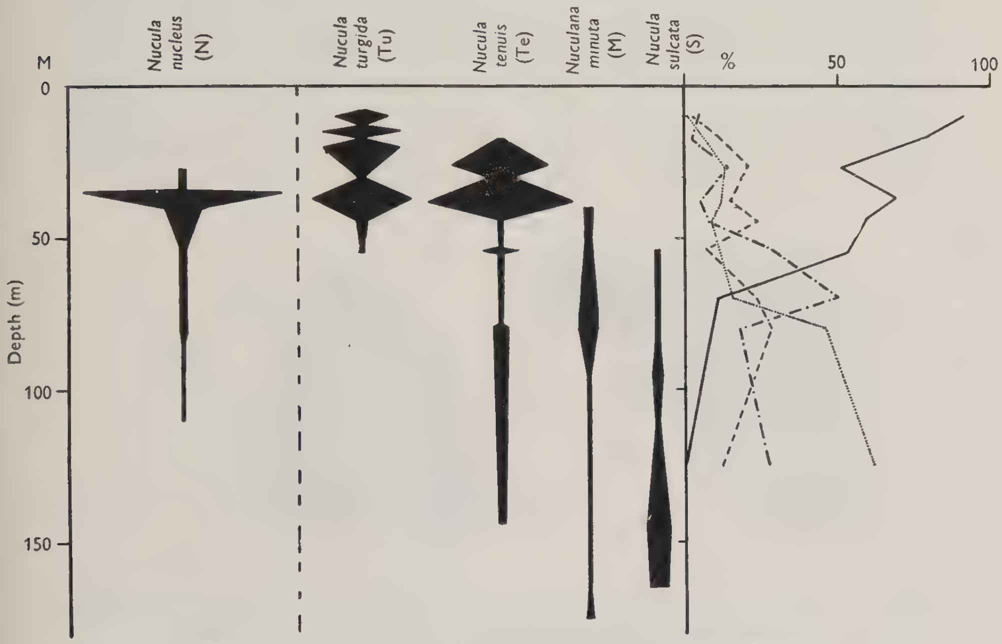
The habitat varies for the different species. They occur on all types of substrata ranging from sand to mud and from sandy-gravel to muddy-gravel.

Nucula turgida was obtained from sands and sandy silts in water ranging in depth from 8 to 100 m, and very occasionally from muds at a maximum depth of 180 m. It is most abundant in sandy-mud (Text-fig. 1). It is present in large numbers in the Cumbrae Deep where there is a small amount of gravel and sand present in the mud. Where *N. turgida* is most abundant it is found together with *Pectinaria belgica*, *Cyprina islandica*, *Abra alba*, *Corbula* (*Aloidis*) *gibba* and *Amphiura filiformis*.

N. sulcata was taken in large numbers in floccular muds such as are present in the Arran Deep. It is found also in muds which contain small quantities of gravel and was taken from the Cumbrae Deep; however, there is evidence that animals from this locality differ in some respects from the rest of the Clyde specimens. It was taken in depths ranging from 60 to 200 m. It occurs together with *Lipobranchius ieffreysii*, *Glossus humanus*, *Amphiura chiajei* and *Brissopsis lyriformis*.

Nucula tenuis is the least common of the British species of *Nucula*. It is most abundant in soft sandy mud but extends into the less floccular muds and thus overlaps the ranges of both *N. turgida* and *N. sulcata*. It was taken from depths ranging from 20 to 150 m. Associated with it is *Thyasira flexuosa* and some of the species above, e.g. *Abra alba*, which can tolerate a fairly wide range of particle sizes.

Nucula nucleus was taken in a few restricted areas within the Clyde such as the Fairlie Channel and the Minard Narrows, where the bottom is a coarse muddy gravel. The associated fauna is similarly restricted in habitat, and the dominant species are *Astarte sulcata*, *A. montagui* and *Dentalium entalis*.



Text-fig. 1. Distribution of species of *Nucula* and *Nuculana* along a traverse from Etterick Bay, Bute, to the Arran Deep compared with the particle size of the substratum. Particle size: >62·5 μ, ——; 62·5–31·2 μ, - - -; 31·2–15·6 μ, –·–·; <15·6 μ, · · ·.

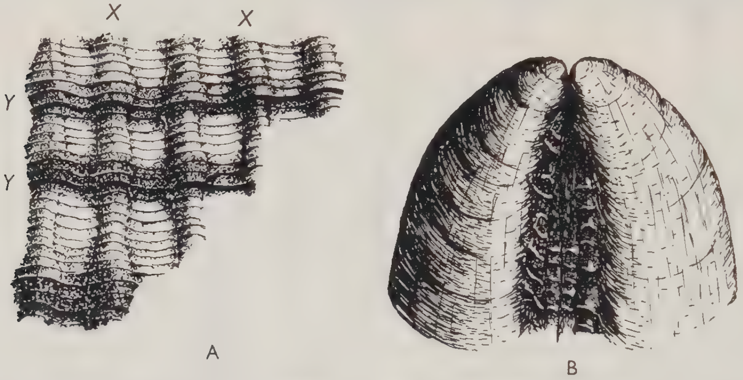
Nucula hanleyi has not yet been taken in the present Clyde survey. It was obtained at Port Erin from a fine sandy gravel at a depth of between 16 and 20 m. It occurs in an offshore fine-gravel community described by Jones (1951).

Nuculana minuta closely resembles *Nucula sulcata* in its habitat range, although it extends farther into the sandy mud grades and is most abundant in a less floccular mud. The associated fauna is the same as that of *N. sulcata*.

The faunal survey has shown that over considerable parts of the Clyde area there is a gradation of particle size with depth. Large particles—rocks, stones, sand—at shallow depths grade to floccular muds in the deeps (see Text-fig. 1). Correlated with this is a sublittoral zonation of the fauna. This is particularly obvious in the case of the Lamellibranchia. The Nuculidae show such a zonation and thus resemble the intertidal Littorinidae. It is where the gradation of the particles is interrupted by shelves and banks of shell gravel that *N. nucleus* is found.

Shell Colour and Characteristics

Shell colour has been variously described as ranging from yellow through various olivaceous hues to grey. Forbes & Hanley (1853) and Jeffreys (1863) have recognized colour differences in different species, but the present observations show that the variation in colour cannot be used as a diagnostic character. For all species there is a background colour of yellow on which a variety of coloured markings may be present. The most common additional colour is purple-grey which is found on the shells of all the British species of *Nucula* and *Nuculana minuta*. This may completely obscure the yellow.

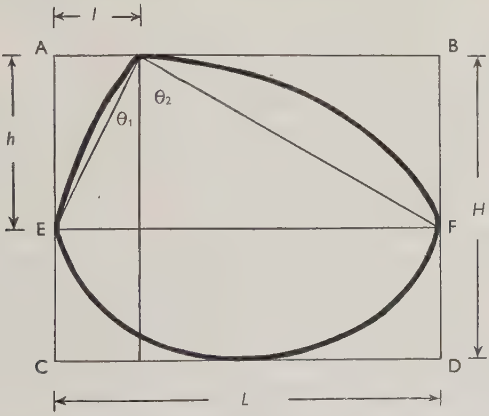


Text-fig. 2. Shell markings of *N. sulcata*. A, shell sculpturing: *X*, striae; *Y*, growth lines. B, anterior dorsal hinge region to show typical transverse corrugations.

The grey colour is rarely present in *Nucula tenuis*, this species usually being clear yellow. In *N. turgida*, as already described (Allen, 1953*a*), it is often in the form of radiate markings from the umbo to the free-margin (see Pl. I). Similar radiate markings occur on *N. hanleyi*, but these are red and, like those of *N. turgida*, vary in width and numbers. This red colour has been observed also on *N. sulcata* taken from the Cumbrae Deep, though here it is not as rays but in patches of varying size. Radiate markings have never been observed in *N. nucleus*, *N. tenuis* and *Nuculana minuta*.

There are differences in the surface of the periostracum, *Nucula turgida* and *N. tenuis* have a very glossy surface, while *N. sulcata* and *N. nucleus* have matt surfaces. *N. hanleyi* and *Nuculana minuta* are intermediate in this respect, although the former is more glossy than the latter. The species with matt surfaces collect a deposit of manganese on their shells while those with a glossy surface do not. *Nuculana* and *Nucula hanleyi* are again intermediate, the deposit never being so heavy.

There are few differences in shell sculpturing. *N. nucleus*, *N. hanleyi* and *N. turgida* have striae radiating from the umbo to the free-margin which may be very slightly decussated by an occasional strongly marked growth line. The growth lines of the latter three species are obvious and the inside edge of the free-margin is crenated. *N. sulcata* is finely decussated (Text-fig. 2A) and in the hinge area anterior to the umbo there are characteristic irregular transverse corrugations (Text-fig. 2B). The inside edge of the free-margin is also crenated. *N. tenuis* and *Nuculana minuta* have no radiating striae. The former shell has a smooth surface with very few growth lines showing while the latter is strongly ridged with growth lines, neither have a crenated inner shell margin.



Text-fig. 3. Diagram to show shell measurements taken. For explanation of symbols see text.

SHELL MEASUREMENTS

Apart from ridges on the gut wall which produce grooved faecal pellets (Moore, 1931*a*), examination of the anatomy did not show any significant differences between the species studied. Comparisons, therefore, have been made from shell features. Measurements were taken of 413 *Nuculana minuta*, 490 *Nucula sulcata*, 227 *N. nucleus*, 2500 *N. turgida* (large numbers taken for examination of the validity of *N. moorei*, see Allen, 1953*a*), 100 *N. hanleyi* and 106 *N. tenuis*.

Shells were placed on a grid ABCD (see Text-fig. 3) so that a line joining the points E and F is parallel to AB and CD (point F in *Nuculana* was taken as the dorsal corner of the posterior end of the shell). The distances H, L, l and h were then measured, and from these the angles θ_1 and θ_2 were calculated. In addition, the greatest width (W) was measured. All measurements were recorded to the nearest 0·1 mm and the angles to the nearest minute. Measurements were taken with the aid of a travelling microscope and vernier calipers.

The mathematical description of the growth and form of the molluscan shell has been discussed by Huxley (1932), Thompson (1942), Lison (1949), and Owen (1953). Quantitative measurement of the specific differences of the British Nuculidae has been considered in relation to the above work and with reference to: (i) growth and form of the generating curve, (ii) the constant angle of the normal axis, (iii) the form of the normal axis, and (iv) the angle of retardation.

Growth and form of the generating curve.[1] Although Owen states that the measurements of 'length', 'height' and 'breadth' do not alone provide a satisfactory means of comparing shell form in different species, they nevertheless provide useful information. In the majority of lamellibranchs, including *Nucula*, shell increments are gnomons and the shape of the generating curve remains constant throughout the post-larval life. The umbo of *Nucula* may be considered as lying at a point on the generating curve and, providing the points E and F lie on a line parallel to AB and CD, the shell measurements L, H, l and h can be compared. Neglecting the curvature, these measurements provide the simplest description of the form of the generating curve. The angles θ_1 and θ_2 are important as they are a resultant of l and $L-l$ and h and further emphasize the specific differences in shell shape.

The constant angle of the normal axis. The constant angle of the lamellibranch is small (approx. 40°). Thompson (p. 743 *et seq.*) points out that it is not easy to measure accurately at such a low value. As in *Nucula*, a tangential component of growth may be present such that the normal axis is no longer a straight line from umbo to free-margin. However, as the tangential component in *Nucula* is small and is of the same order in all species, the ratio $H/\frac{1}{2}W$ has been used to compare the concavity of the valves, i.e. the constant angle.

The form of the normal axis. This is turbinate for all *Nucula* species. The measurement of the degree of turbination is difficult and no accurate determination was made. Observation of *N. turgida* and *N. hanleyi* with radial shell markings and of those species with striae indicates that the degree of turbination is of the same order in all.

Angle of retardation. As the umbo approximates to a point on the generating curve in all the Protobranchiata, the angle of retardation, i.e. the retardation of growth of the inner as compared with the outer whorl, approaches infinity and thus can be neglected.

In addition, age and growth rates and hinge teeth numbers are compared in the species studied.

COMPARISON OF SHELL SHAPES

Generating curve

Comparison of the overall measurements of height (H) and length (L) shows there is very little difference between the five species of *Nucula*. Thus mean shell heights calculated for a length of 1 cm are as follows (length was chosen because the growth increments in length are almost constant for the British species of *Nucula*): *N. nucleus* 0·83 cm, *N. sulcata* (Cumbrae Deep) 0·81 cm, *N. hanleyi* 0·80 cm, *N. turgida* 0·78 cm, *N. sulcata* (Arran Deep) 0·78 cm and *N. tenuis* 0·76 cm. Comparisons of the individual variations in the overall measurements of height and length emphasize the fact that the measurements overlap each other too much to show any significant difference between the species. However at high values the measurements of *N. nucleus* and *N. tenuis* (the highest and lowest respectively in relative height measurements) when considered by themselves are nearly distinct. There is also a distinction between *N. sulcata* from the Cumbrae Deep and the Arran Deep. This is also indicated in other measurements, and the possible explanation will be discussed later (see p. 465).

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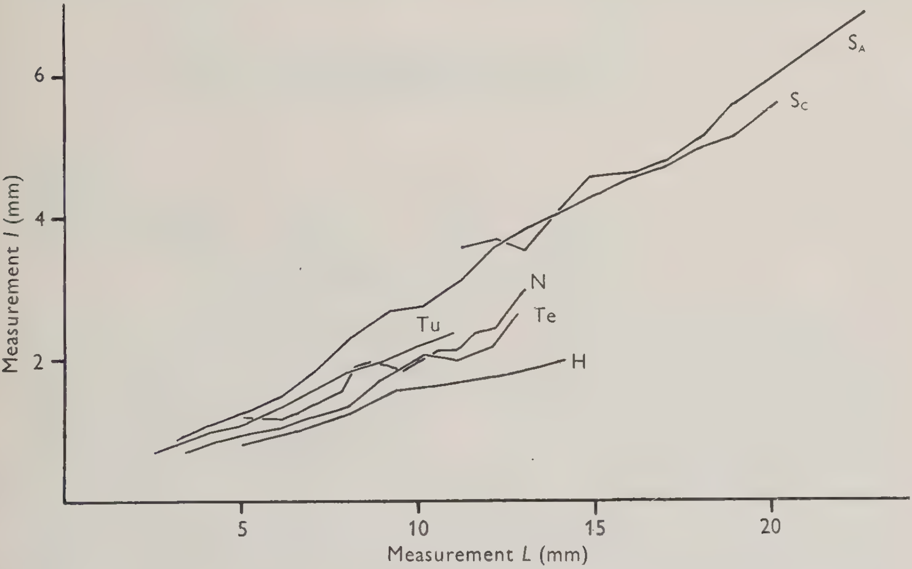
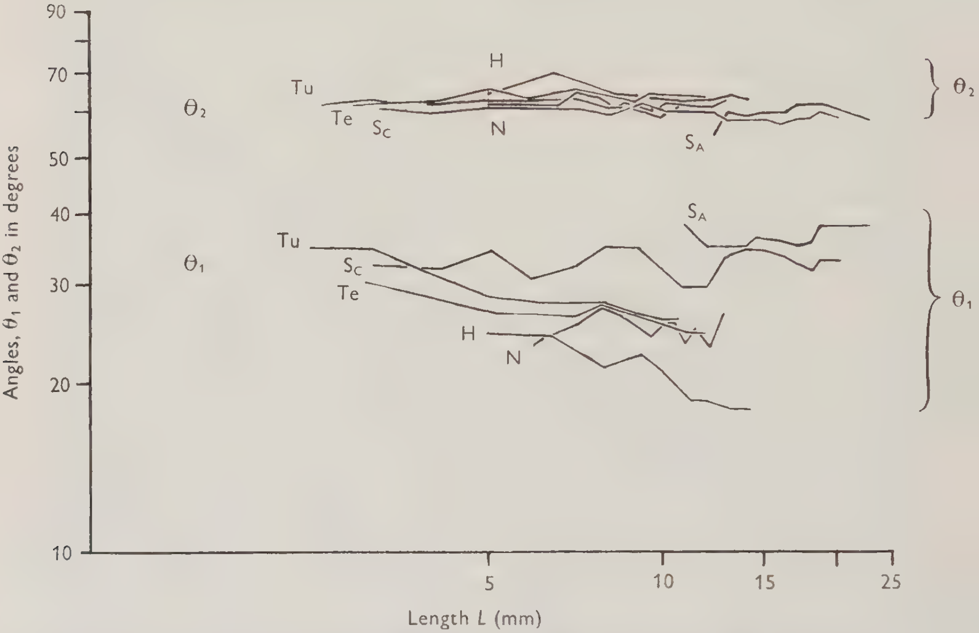
[1] The nomenclature proposed by Owen (1952, 1953) has been used.



Text-fig. 4. Comparison of the mean shell measurements, *l*, plotted against length (*L*). H, *Nucula hanleyi*; N, *N. nucleus*; S, *N. sulcata*; Tu, *N. turgida*; Te, *N. tenuis*; A, Arran Deep; C, Cumbrae Deep.

Although the overall measurements show few differences the angles θ_1 and θ_2 need not be the same for all species. Reference to Text-fig. 4 shows that specific differences for the value l are marked and that *N. hanleyi* has the lowest value for l and *N. sulcata* the highest. Here, the measurements of *N. nucleus* and *N. tenuis* lie much closer together, the latter having the lower values for l. These slight differences in l and H (*N. tenuis* having a lower value for both measurements) make the values of θ_1 for both these species approximately equal, while the values for θ_2 are distinct. This explains why *N. tenuis* appears elongate when compared with the other species (Text-fig. 5).

θ_1 and θ_2 are the best measurements for comparing the generating curves as they take into account both measurements for height and length. Values of θ_1 in Text-fig. 5 further show that *N. sulcata* and *N. hanleyi* are distinct while values for the other species are nearly identical. Although differences in $L-l$ will not be so clearly reflected in θ_2 when this is in the region of 60°, Text-fig. 5 shows that values of θ_2 for *N. nucleus* approximate to those of *N. sulcata* while those of *N. tenuis* lie close to those of *N. hanleyi*. Again there are differences in the measurements of *N. sulcata* from the Cumbrae Deep and those from the Arran Deep. Whereas the species other than *N. sulcata* with low values for θ_1 have high values for θ_2, *N. sulcata* from the Arran Deep with a larger angle θ_1 than *N. sulcata* from the Cumbrae Deep, also has a larger angle θ_2 than the shells from the Cumbrae Deep.



Text-fig. 5. Comparison of the mean shell angles θ_1 and θ_2, double-log plot. Abbreviations as given under Text-fig. 4.

Constant Angle

Reference to Table I shows that the differences in the constant angle are very slight. However, they do bear out the field observations that *N. nucleus* has the greatest spiral angle and *N. tenuis* the smallest.

Discussion and Conclusions on Shell Shape

It is convenient at this stage to summarize the above data and attempt to correlate the differences in shell measurements with the differences in habitat. Table I summarizes the shell measurements of the species of *Nucula* which are calculated for a shell length of 1·0 cm. Although there are few differences in the overall shell measurements, the species can be distinguished by considering the measurements in relation to the position of the umbo. Studies on normal variation show that there is an overlap of the measurements, but consideration of all the measurements, particularly when these are taken in conjunction with other characters (see p. 471), show that the species of *Nucula* can be distinguished from each other. The most important shell measurements are the angles θ_1 and θ_2 for they are the resultant of l and $L-l$ and any slight difference in height. The effect of the slightly smaller height measurements of *N. tenuis* has meant that while θ_1 for this shell is of the same order as that of *N. turgida*, its value for θ_2 is slightly larger than that of *N. hanleyi*. This explains the characteristically elongate appearance of *N. tenuis*. *N. turgida* and *N. nucleus* are the only species that cannot be readily separated by shell measurements, although *N. nucleus* has the highest measurement of height and the greatest spiral angle. *N. sulcata* shows differences in measurements at different localities. Similar differences have been shown for *Chlamys septemradiata* taken from different regions of the Clyde (Allen, 1953*b*). Differences between *Nucula sulcata* from different localities and the shell differences between the other species are apparently correlated with differences in their habitat. *N. sulcata* living in soft floccular mud has a large measurement for l, while *N. hanleyi* in firm sandy-gravel has a small measurement for l. The other species of *Nucula* are intermediate both in type of substratum and measurement l. The mud of the Cumbrae Deep is more compact than that of the Arran Deep and has a small amount of sand and gravel mixed with it. Thus some differences might be expected between *N. sulcata* taken from these localities. Thus differences in shell shape may have arisen because those with a small l value may be better adapted for movement in firm substrata.

TABLE I. MEAN SHELL MEASUREMENTS CALCULATED FOR $L = 1{\cdot}0$ CM

(Linear measurements in centimetres)

	l	$L-l$	h	H	W	$H/\frac{1}{2}W$	θ_1	θ_2
Nucula tenuis	0·19	0·81	0·40	0·76	0·41	3·76	26° 31′	63° 40′
N. turgida	0·23	0·77	0·43	0·78	0·45	3·46	26° 56′	60° 41′
N. sulcata (C.D.)	0·28	0·72	0·43	0·81	0·44	3·68	32° 54′	58° 45′
N. sulcata (A.D.)	0·30	0·70	0·41	0·78	0·43	3·63	36° 18′	59° 13′
N. nucleus	0·21	0·79	0·44	0·83	0·49	3·38	25° 13′	61° 06′
N. hanleyi	0·15	0·85	0·42	0·80	0·44	3·63	19° 42′	63° 26′

C.D., Cumbrae Deep. A.D., Arran Deep.

AGE AND GROWTH

The histograms in Text-fig. 6 show that the species of the Protobranchiata examined have definite size-groups which are in all probability year-groups (see Allen, 1953*a*). *N. turgida* and *N. sulcata* were found to have ripe sperm and eggs in January and February but no successful fertilizations were carried out. *N. nucleus* differs from the other species in that the size-groups indicate that it either grows at half the rate of other *Nucula* species or that there are two breeding periods annually. Lebour (1938) states that the breeding period is from spring to autumn (April to November). This is longer than the other species. There are probably two maxima, a view that is supported to some extent by the fact that Dr Lebour was unable to carry out artificial fertilization in August. Assuming that the size-groups are year-groups (in *N. nucleus* two size groups per year) then the maximum ages attained by the species are as follows: *N. turgida* 12 years, *N. tenuis* 13 years, *N. nucleus* 13 years, *N. hanleyi* 14 years, *N. sulcata* 20 years and *Nuculana minuta* 17 years.

The increments of total length and total height are nearly the same for all species, this being particularly true of the length increment (see Table II below).

TABLE II. MEAN YEARLY SHELL INCREMENTS

(Measurements in mm)

	L	*l*	*L−l*	*h*	*H*
Nucula turgida	0·94	0·19	0·75	0·43	0·75
N. tenuis	0·96	0·18	0·78	0·42	0·76
N. hanleyi	1·01	0·13	0·82	0·47	0·83
N. sulcata (C.D.)	0·96	0·27	0·69	0·40	0·76
N. sulcata (A.D.)	0·96	0·28	0·68	0·38	0·70
N. nucleus	0·94	0·21	0·73	0·41	0·80
Nuculana minuta	0·98	0·52	0·46	0·28	0·57

Nucula hanleyi is an exception but this is offset by a correspondingly high growth rate in height. *N. nucleus*, the other species living in gravel, also has a high height increment while *N. sulcata* from the floccular mud of the Arran Deep has the lowest. The growth rate remains practically constant throughout the life of the species. *Nuculana minuta*, although being a member of a different family with marked differences in shell shape, has the same growth increment of length with similar year-groups (see Text-fig. 6).

Shell weights have been compared and these show that the curves for increase of shell weight with length are similar (Text-fig. 7). *Nucula hanleyi* differs slightly in that the initial rate is slightly less than *N. turgida* and *N. nucleus*. This increases between 8 and 10 cm length so that at high

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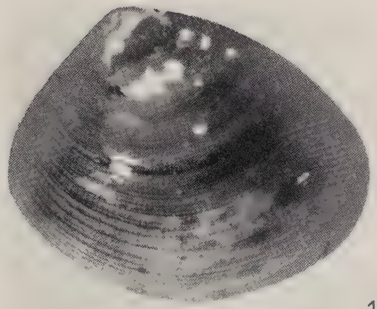
EXPLANATION OF PLATE I

Lateral views of the right valve of the five British species of *Nucula*. 1, *N. sulcata* (Cumbrae Deep); 2, *N. sulcata* (Arran Deep); 3, *N. nucleus*; 4, *N. turgida*; 5, *N. hanleyi*; 6, *N. tenuis*.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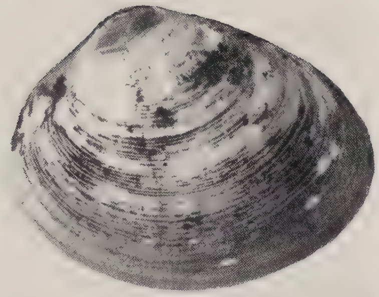
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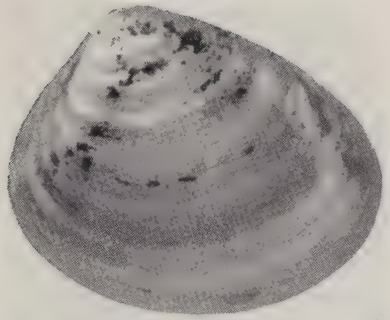
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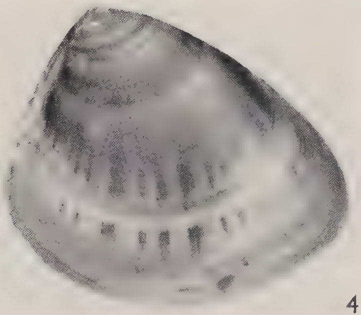
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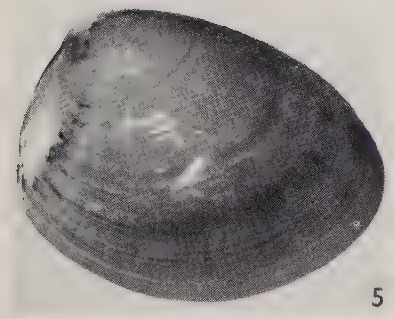
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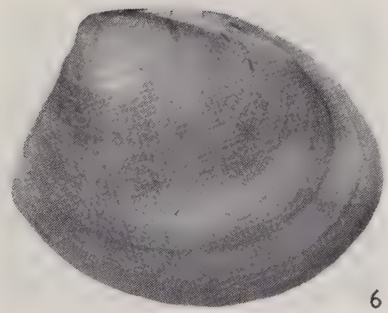
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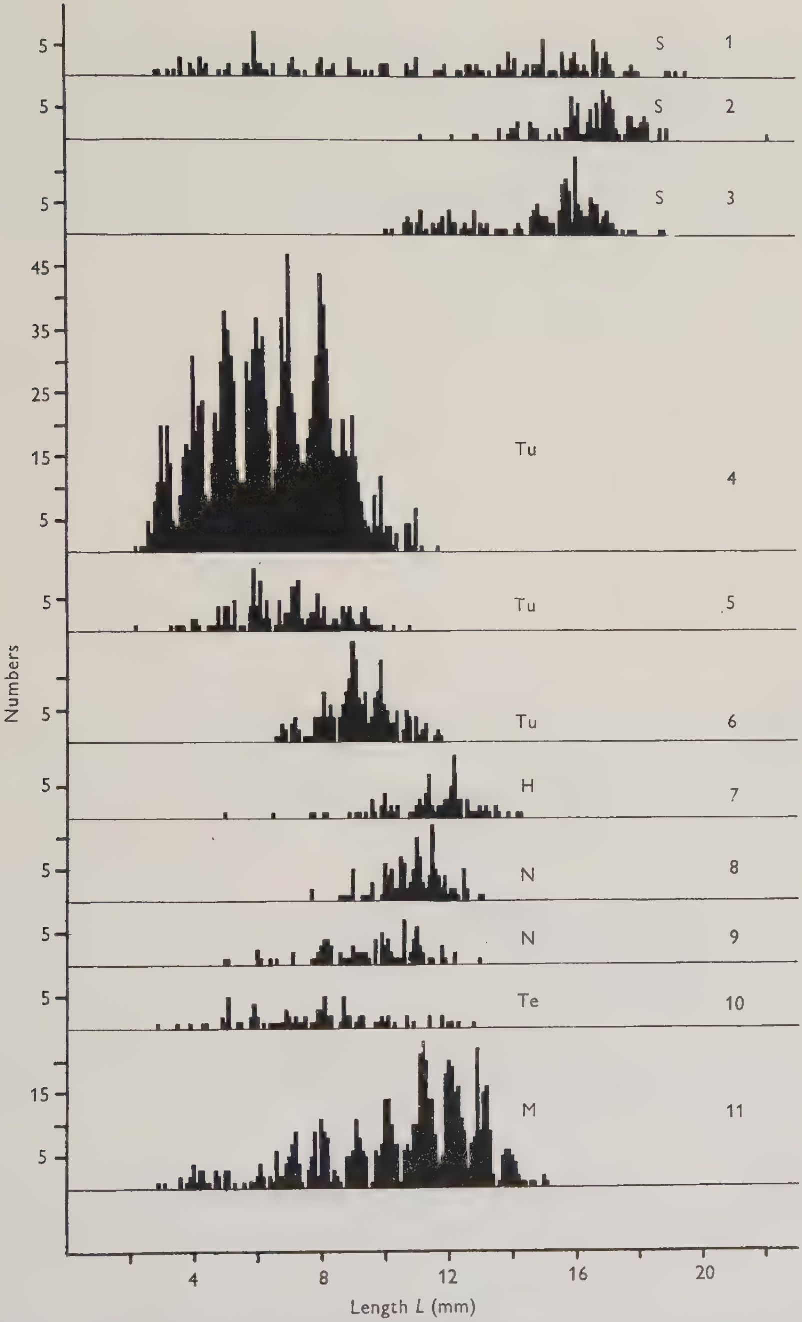
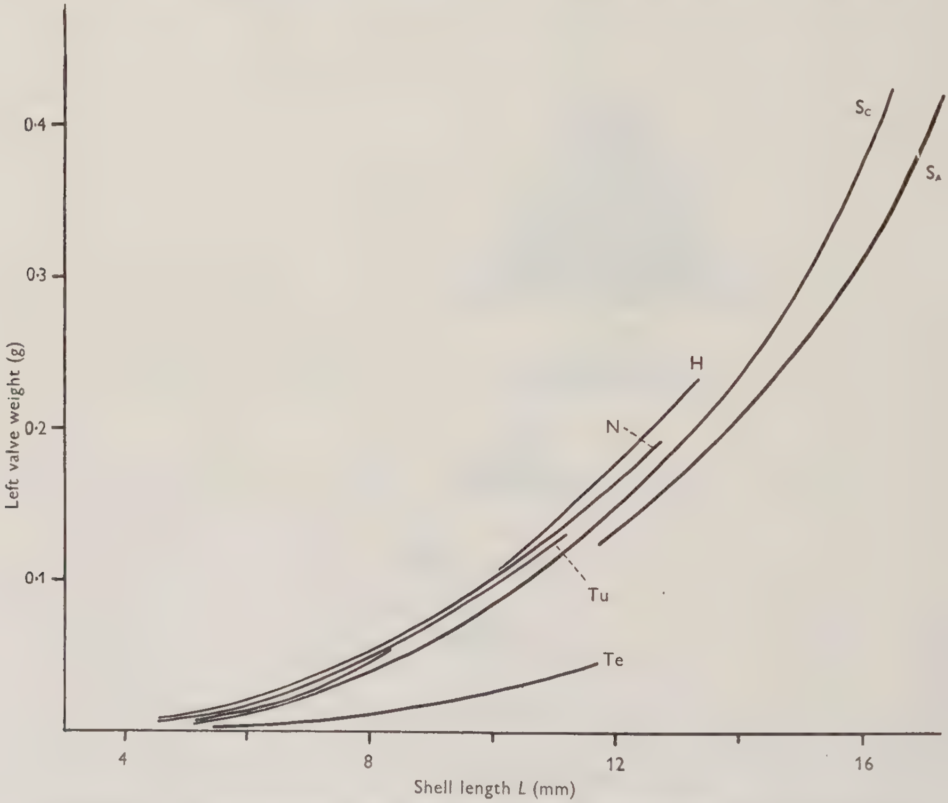


Text-fig. 6. Population histograms of the largest samples taken. 1, *Nucula sulcata*, Cumbrae Deep, July 1951. 2, *N. sulcata*, Arran Deep, July 1951. 3, *N. sulcata*, Arran Deep, Aug. 1951. 4, *N. turgida*, Cumbrae Deep, Oct. 1950. 5, *N. turgida*, Cumbrae Deep, June 1951. 6, *N. turgida*, Minard Narrows, July 1951. 7, *N. hanleyi*, Port Erin, Jan. 1952. 8, *N. nucleus*, Fairlie Channel, Dec. 1948. 9, *N. nucleus*, Minard Narrows, Aug. 1951. 10, *N. tenuis*, Etterick Bay, July 1949. 11, *Nuculana minuta*, Kilbrennan Sound, Aug. 1951.

measurements it is the heaviest shell. These observations indicate that shell weight, also, can be correlated to habitat. Thus mud-dwelling species where the bottom is soft have the lightest shells while those from harder substrata and at low depths where the wave action is the strongest have the heaviest shells. *N. tenuis* differs in that it has a very light shell and possibly for this reason is able to extend its range into soft deep water muds. *N. sulcata* from the Arran Deep has a lighter shell than specimens from the Cumbrae Deep.



Text-fig. 7. Increasing weight of a single valve (left) plotted against length (L) in the five British species. Abbreviations as given under Text-fig. 4.

HINGE TEETH

Although there is some individual variation (see Text-fig. 8) species of *Nucula* differ in numbers of hinge teeth. These specific differences may be correlated with differences in shell weight and with differences in l and $L-l$. The species with heavier shells have high teeth numbers. The heavier shells also come from hard substrata and have low values for l. Thus the posterior hinge region is shorter than in the species from soft substrata and the numbers of teeth in this region will be restricted by lack of space. Specific differences in the length of the relatively long anterior hinge are not great enough to have much effect on teeth numbers. Shell weight rather than limiting space has the greater effect on teeth numbers (see Text-fig. 8). *N. sulcata* with the longest value of *l* has low numbers of teeth and it is only in the case of *N. hanleyi* that a low value for *l* appreciably lowers the number of posterior teeth. *N. tenuis* with the very fragile thin shell has the least number of teeth.

Hinge numbers increase with increasing age and there are specific differences in the rate at which they are laid down. This rate is not the same anterior and posterior to the hinge, the posterior teeth being laid down at a slower rate. *N. sulcata* from the Arran and Cumbrae Deeps are almost identical in teeth numbers, with a slight tendency for fewer in the Arran Deep shells.

TABLE III. AVERAGE INCREASE IN SHELL LENGTH (MM) FOR EACH ADDITIONAL TOOTH

	Anterior	Posterior
Nucula sulcata	1·20	1·65
N. tenuis	1·15	1·40
N. turgida	0·85	0·95
N. nucleus	0·60	0·95
N. hanleyi	0·70	1·15

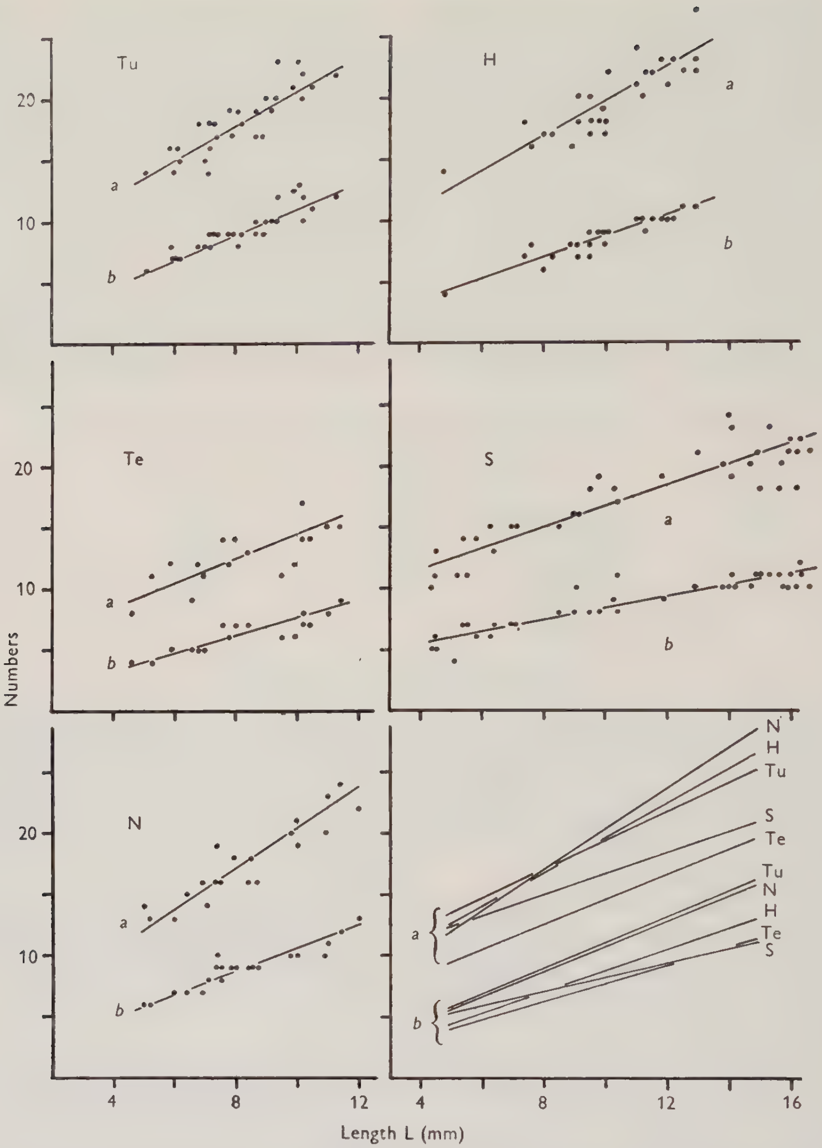
DISCUSSION AND CONCLUSIONS

The British species of *Nucula* occur commonly on a variety of sublittoral substrata. They are so similar that it has been found difficult to distinguish between them. *N. turgida* and *N. moorei* have recently been shown to be the same species (Allen, 1953 *a*) while Moore (1931 *a*) has suggested that *N. hanleyi* and *N. nucleus* are identical. Careful examination shows that five British species can be recognized.

Species of the Protobranchiata have successfully invaded most sublittoral substrata, the main exception being rocky bottoms. Each species is restricted to a particular type of substratum. Within the Clyde, where, over much of the area there is a gradation of particle sizes with depth, they show a sublittoral zonation.

Shell colour is similar in all species. *N. turgida* and *N. hanleyi* may have coloured rays from the umbo to the free-margin varying in width and extent. There is little shell sculpturing, that of *N. sulcata* being the most pronounced. *N. tenuis* differs from the other species in that it has no crenations along the inner edge of the free-margin of the shell. There are differences in the degree of gloss of the surface of the periostracum ranging from *N. tenuis* with a high gloss to *N. sulcata* with a matt surface.

Shell measurements were taken and studied in the light of recent work on the growth and form of the lamellibranch shell. There is little difference in the overall shell measurements of length and height but the measurements



Text-fig. 8. The variation and comparison of the numbers of anterior (*a*) and posterior (*b*) teeth at different values of length (*L*). Abbreviations as given under Text-fig. 4.

l and $L-l$ are important in distinguishing between the species. The angles θ_1 and θ_2 are also important as they are the result of both differences in l and $L-l$ and height. Thus the slightly lower measurements in height of *N. tenuis* and *N. sulcata* from the Arran Deep are reflected in the angles θ_1 and θ_2 and emphasize differences already shown in l and $L-l$. Differences in shell form may be correlated with differences in habitat. Thus species in hard substrata with large particles (*N. hanleyi*) have smaller measurements for θ_1 and l than those from soft mud (*N. sulcata*). This may be correlated with the fact that a greater pull of the foot is necessary for movement through the harder ground. It has been shown that the weight of shell in the species living in mud is less than in species living in sand and gravel. This is reflected in the number of shell teeth present, i.e. fewer in species from mud. This is probably associated with the fact that shells from sand and gravel tend to be more robust, particularly in the Clyde Sea Area where they are in shallow water and will be affected more by wave action.

Not only can specific differences be related to differences in substratum but species themselves can differ in different habitats. This is very well shown in *N. sulcata*.

N. nucleus and *N. hanleyi* are not one and the same species as was suggested by Moore in his study on faecal pellets. On the other hand, *N. turgida* and *N. moorei*, recently shown to be the same species, were separated by Winckworth on supposed differences in the faecal pellets as demonstrated by Moore. It must be concluded that differences in pellets cannot be used by themselves for the identification of *Nucula* species.

The specific differences have been summarized in Table IV.

TABLE IV. COMPARISON OF THE SPECIES OF *NUCULA*

	N. hanleyi	*N. nucleus*	*N. turgida*	*N. tenuis*	*N. sulcata*
Habitat	Sandy gravel	Muddy gravel	Muddy sand	Sandy mud to mud	Mud
Shell colour, etc.	Red rays, periostracum fairly glossy, inner margin crenated	No rays, periostracum matt, inner margin crenated	Purple-grey rays sometimes present, periostracum glossy, inner margin crenated	No rays, periostracum glossy, inner margin not crenated	No rays, periostracum matt, inner margin crenated, slightly decussated with irregular transverse corrugations above ant. hinge
Shell shape, measurements, in parentheses in mm, mean values given	Lowest measurement for l (0·15) and θ_1 (19° 42′)	Differs only slightly from *N. turgida* but has highest measurement of H (0·83) and constant angle (3·38)	All measurements are intermediate to other species	Lowest measurement of H (0·76) and largest angle θ_2 (63° 40′)	Highest measurement for l (0·30) and θ_1 (36° 18′)
Maximum age, growth, etc.	14 years, greatest growth rate and heaviest at lengths above 1 cm. One breeding period/year	13 years, two breeding periods/year	12 years, one breeding period/year	13 years, very light and fragile shell with very low hinge teeth numbers, one breeding period/year	20 years, one breeding period/year

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J. Mar. biol. Ass. U.K. (1954) 33, 473–496
Printed in Great Britain

THE BREEDING OF *BALANUS PORCATUS* (DA COSTA) IN THE IRISH SEA

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(Text-figs. 1–10)

Balanus porcatus (da Costa) is a widely distributed northern species, being found in the Arctic Ocean as far as 80° N., and in the northernmost extensions of the Atlantic and Pacific Oceans. Its southern limits are closely related to water temperatures. Whereas on the east coast of America, where the Labrador current flows southward, it extends to Long Island (lat. 40° N., see Pilsbury, 1916), on the west European coast it scarcely penetrates the English Channel (lat. 51° N.). It is found in some abundance however in the North Sea, the Skagerrak and Kattegat (Krüger, 1927), the Irish Sea, and off the west coast of Scotland and Ireland. The present survey is probably representative of its breeding habits in regions near the southern limits of its range.

In the Irish Sea it is generally found from 3 to 20 fathoms, usually in association with *Modiolus modiolus* (L.) or *Pecten maximus* (L.). This association exists probably because these molluscs provide much of the suitable substrata offshore, for the barnacle is also found on stones, pieces of coal, etc., when these happen to be dredged in the vicinity of the *Modiolus* beds. It occurs occasionally on rocks at low-water mark and at higher levels in the intertidal zone: for example a few specimens have been encountered at mid-tide level on rocks thickly draped with *Ascophyllum nodosum*, and also on the fronds of *Ascophyllum* itself at Church Island Reefs, Menai Straits.

COLLECTION OF SAMPLES

Most of the samples forming the basis of this study were obtained from a ground in Beaumaris Bay at the eastern entrance to the Menai Straits between Puffin Island and the east side of the Dutchman bank, at 3–5 fathoms. The samples were stripped off *Modiolus* shells collected in an otter trawl. Each haul swept a length of about 1 mile, and generally brought in about a hundred *Balanus porcatus*. Of these, usually about half were isolated specimens, one only occurring on each *Modiolus* shell, while the other half occurred in groups of two or more barnacles on each shell. The high proportion of isolated individuals indicated that the ground was not subject to heavy settlements of this species, but the large size of the barnacles showed that the conditions were favourable for survival and growth.

The only abundant forms whose presence was clearly deleterious to the barnacles were *Alcyonium digitatum* L. and *Cliona celata* Grant. Barnacles completely covered by *Alcyonium* were sometimes dead, or moribund, or, if alive, had very poorly developed ovaries and were therefore excluded from the survey. *Cliona* infected the shells of many older barnacles and completely filled the majority of dead shells. The only other barnacles observed in the samples were occasional individuals or small groups of *Balanus crenatus* Brug. and *Verruca stroemia* (O. F. Müller).

Samples were also kindly taken for me by the staff of the Marine Biological Station, Port Erin. These served as corroborative evidence. Most of them were taken south and west of Port Erin Bay and Chicken Rock, where *Balanus porcatus* occurs mainly on *Pecten* shells in association with *Balanus crenatus*. It is also occasionally found on *Modiolus* shells south-west of Port St Mary in association with *Balanus hameri*. The specimens from Port Erin differed from those from Beaumaris Bay in being smaller and more closely packed on the shells and, moreover, in being occasionally infected by the parasitic isopod *Hemioniscus*, presumably *H. balani* (Bate), which has been reported in several other cirripedes besides its normal host *Balanus balanoides* (L.) (Crisp, 1951; Sandison, 1954). These differences are shown in Table I. The absence of isopod parasites from *B. porcatus* dredged in Beaumaris Bay was not unexpected since the incidence of *Hemioniscus* in *Balanus balanoides* was also low on the surrounding shores.

TABLE I. DIFFERENCES BETWEEN SAMPLES OF *BALANUS PORCATUS* ON SHELLS OF *PECTEN MAXIMUS* TAKEN SOUTH-WEST OF PORT ERIN AND ON *MODIOLUS MODIOLUS* FROM BEAUMARIS BAY

	Port Erin samples	Beaumaris Bay samples
Number measured	61	271
Mean basal diameter (mm)	16·1	26·4
Percentage with basal diam. > 30 mm	0	29
Numbers examined for *Hemioniscus*	61	831
Number infected by *Hemioniscus*	4	0
Percentage infection	6·6	0

The barnacles were removed from their substrate in the laboratory with a strong knife and were generally examined while fresh; a few samples were perforce examined after storage in 5% formaldehyde in sea water.

On opening the barnacle, it was immediately clear whether the ovary had been discharged. Fertilized eggs formed two conspicuous masses lying in the mantle cavity on either side of the body, as illustrated by Moore (1935) for *B. balanoides*. Unfertilized eggs, on the other hand, were found within the tubules of the ovary, immediately within the calcareous basis, and had the appearance of a diffuse, fatty tissue, creamy yellow in colour. As fertilization in this species occurs in each individual only once a year, during the month of February, the frequency of sampling was intensified in January, and throughout the ensuing period when the embryos were developing in the paired egg sacs, until their release into the sea in April and May. The period from January to May not only witnesses the most significant changes in the female gonads, but striking changes take place in the male organs, which were also studied. Sampling was continued, but less frequently, throughout the rest of the year.

THE MALE REPRODUCTIVE ORGANS

The testes, like those of other cirripedes (Darwin, 1854), consist of a mass of club-shaped diverticula lined with germinal epithelium. They communicate directly with the paired vesiculae seminales and discharge spermatozoa into them. In an early state of development the lobes of the testis are cylindrical, few in number, and devoid of sperm, hence perhaps readily overlooked; but when fully developed they are extensive and clearly visible through the cuticle. The degree of development of testis tissue in each individual examined was assigned to one of the following classes: (i) absent; (ii) poorly developed, visible only on dissection or teasing out; (iii) moderately developed, occupying a small part of the body and just visible through the cuticle; (iv) well developed, occupying a considerable part of the body and clearly visible from outside. In order to pool the information in a given sample and to represent more simply the probable state of development of the whole population on that date, these classes were scored 0, 1, 2 and 3 respectively, so that the average score taken over all individuals of the sample could be used as an index.

In these investigations only individuals of diameter exceeding 10 mm were included, smaller ones being immature; most samples consisted of from ten to twenty mature individuals.

The vesiculae seminales are paired fusiform sacs lying close to the dorsal surface visible through the cuticle as two prominent white patches, except when they are atrophied. When fully developed they become extremely bloated and turgid because of the great quantity of seminal fluid which they contain, and their elastic walls readily cause the discharge of this fluid should the ducts uniting them with the intromittent organ or penis become severed. When they are full the seminal fluid can often be seen extending to the lumen of the penis. The development of the vesiculae seminales was assessed by a method similar to that used for the testes, the classes being: (i) absent, or present as thin strands of tissue; (ii) poorly developed, sacs small, shrivelled, with a little sperm; (iii) moderately developed, sacs cylindrical, of diameter about equal to that of the gut; (iv) well developed, sacs large and bloated, exceeding the gut in diameter.

The penis is a highly extensible and muscular organ, arising medially between the most posterior cirri. It bears annular thickenings in the cuticle, and sensory setae at the tip. Its lumen is continuous with those of the vesiculae seminales, the ducts of which unite just before entering it. In a few individuals of each sample the contracted penis was measured, using a binocular microscope provided with a calibrated micrometer eye-piece. As the penis is readily stretched, great care was taken to treat all samples similarly during measurement. The length of the penis is a function of the size of the individual; therefore the diameter of the basis of each individual was recorded at the same time. If all the results of penis measurements throughout the year are grouped according to the size of the individual bearing the penis, the relation illustrated in Fig. 1 is obtained. The mean penis length is here plotted against the diameter of the basis, with upper and lower limits showing the standard error in the penis length associated with each point. Although there is a wide variation in length for individuals of each size-group,

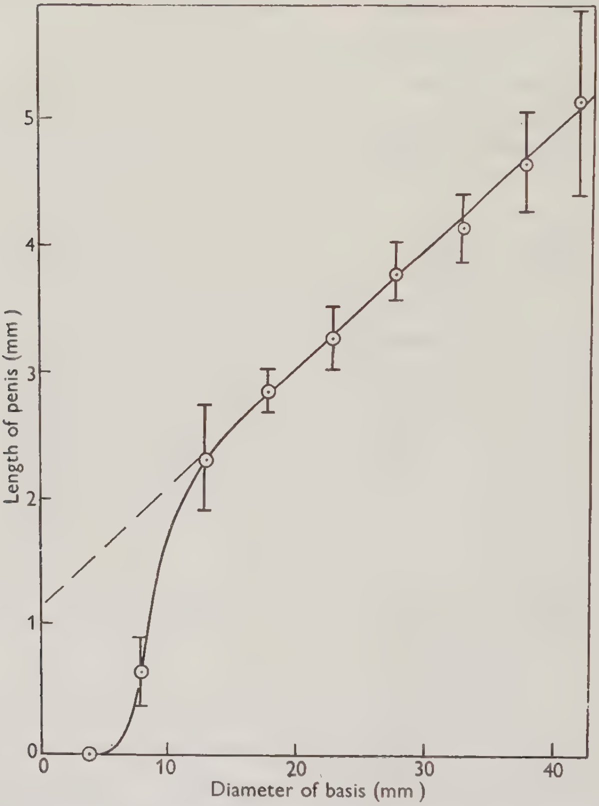

Fig. 1. Relation between the mean lengths of penis (vertical axis) and the basal diameter (horizontal axis) in *B. porcatus*. The vertical lines represent the standard error associated with each point.

it is clear that the growth of the penis is very rapid during the period in which the basal diameter increases from 6 to 10 mm. It is probably even more rapid than appears from the graph since this represents a mean of measurements on numerous individuals which will probably vary in the size-range over which the rapid growth of the penis occurs. If so, such variations would have the effect of spreading the rapid increase in mean length over a wider range of size than would be characteristic of the growth of an individual penis. The basal diameter at which the penis shows this heterogonic growth is attained towards the latter part of the first year of life, and probably takes place in the majority of individuals shortly before copulation is imminent. After the attainment of a basal diameter of 14 mm the mean growth of the penis in relation to basal diameter is approximately constant, the equation relating its length (l) to the basal diameter (d) being $l=(1\cdot10+0\cdot095d)$ mm. In plotting seasonal changes the mean penis length was therefore corrected for variations in size of the individuals in the sample by means of the above equation, each record being reduced to a standard basal diameter of $d=20$ mm. The mean reduced length was then employed as an index of development.

SEASONAL CHANGES IN THE MALE REPRODUCTIVE ORGANS

Fig. 2 illustrates the seasonal changes in the testes, vesiculae seminales, and penis, the index of each being plotted on the vertical axis against the time of year on the horizontal.

Prior to copulation in early February the male reproductive organs attain their fullest development. The vesiculae seminales occupy a large part of the body space, having steadily increased in size since the preceding summer. They reach their maximum size at the time of copulation. The penis is also enlarged at this time, measuring in some individuals as much as 10 mm, and being capable of extension to some two or three times this length. The testes, which also occupy a prominent part of the body space, reach their fullest development a little earlier, and have already begun to decline by the time copulation takes place. After fertilization a marked recession, heralded by rapid degeneration of the testes, affects all the male organs. The penis shrinks, the vesiculae seminales wither into two faint tubes lying dorsal to the gut, and the testes become transparent and devoid of sperm before gradually disappearing.

During April, however, regeneration of the testes begins and within a few weeks these organs are almost reconstituted; indeed the new growth can often be seen to have commenced before the older tissue has been resorbed. Concurrently, the vesiculae seminales are restored and become gradually refilled with sperm. The size of the penis, however, does not increase until later in the year. Thus the slight expansion of the penis in mature individuals and the rapid initial growth in first-year barnacles occur at about the same time.

The decline in the male reproductive organs does not depend upon discharge of accumulated sperm, nor upon the discharge of ova following copulation with other individuals, for it occurs on an identical scale in isolated individuals which cannot inseminate and which retain an unfertilized ovary, self-fertilization not taking place in this species (p. 483). Table II lists the indices of the development of the male organs for parallel sets of solitary unfertilized individuals and for normal adjacent individuals taken during this period and demonstrates that there is no appreciable difference in behaviour.

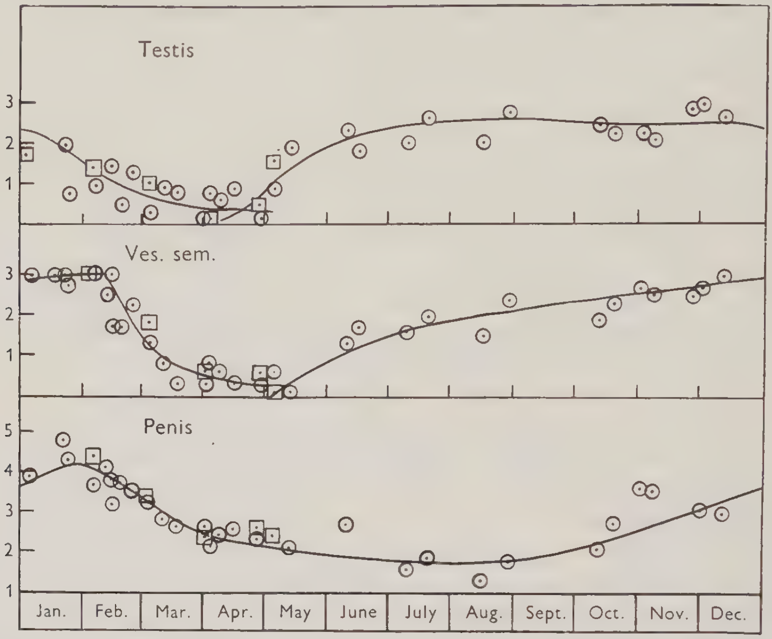

Fig. 2. Seasonal changes in development of penis (bottom curve), vesiculae seminales (middle curve) and testis (top curve). Penis length is given in mm. For the methods of assessment of these organs see pp. 475–7. ⊙, adjacent individuals; ⊡, isolated individuals.

TABLE II. PARALLEL DEGENERATION OF MALE ORGANS IN ADJACENT AND ISOLATED INDIVIDUALS DURING THE PERIOD FEBRUARY TO MAY

	Testis		Vesiculae seminales		Penis	
Date	Adjacent	Isolated	Adjacent	Isolated	Adjacent	Isolated
6. ii. 52	0·95	1·4	3·0	3·0	3·65	4·4
4. iii. 52	0·3	1·1	1·3	1·8	3·25	3·4
1. iv. 52	0·1	0·1	0·3	0·6	2·6	2·4
5. v. 52	0·9	1·6	0·6	0·1	2·55	2·45

The Female Reproductive Organs

The gross development of the ovary of each individual in a sample was classified, scored, and pooled to give an index, following the same general procedure described above. The ovary was classed as: (i) absent, no ovary visible; (ii) poorly developed, only a thin layer of ovarian tissue in the basal membrane; (iii) moderately developed, ovary apparently filling about a third of the mantle cavity; (iv) well developed, ovary apparently filling the greater part of the mantle cavity. A small piece of ovarian tissue was taken where possible and teased out on a slide in a drop of sea water, the size of several of the largest ova present being then measured with a calibrated micrometer eyepiece.

During the breeding season the number of individuals bearing fertilized egg-masses was recorded as a percentage of the total number examined, somewhat larger samples being employed for this purpose than were used in determining ovarian development.

Development and Fertilization of Ova

The ova are of large size compared with those of other British species (Table III), being exceeded only by those of *B. hameri*. In the ovarian tubules they are compressed to a polygonal shape, but when ripe they round off immediately upon release into sea water, where, as would be expected, they may increase somewhat in size if the water is hypotonic and decrease if it is hypertonic. When fertilized they are at first only slightly compressed into an ellipsoid by the egg-shaped membrane which is formed around them. Calculations of the relative volume prior to and after fertilization (ab^2/r^3), treating the egg as an ellipsoid, show that there is a slight reduction in volume when the ripe egg becomes fertilized and is surrounded by an egg case. This appears to be general in several species of cirripedes (Groom, 1894) and is known to occur in other animals (Glazer, 1914; Okkelberg, 1914). It seems likely that just after copulation the eggs are discharged via paired oviducal openings at the bases of the first cirri; these openings become swollen and glandular at the breeding season and may be responsible for secreting the matrix which subsequently hardens and holds together the eggs within each of the egg masses. A moult probably occurs at the time of copulation, as in some other arthropods (Jurine, 1820; Höglund, 1943; Burkenroad, 1947; Schöbl, 1880) since at this time many cast skins appear in the plankton.

Fig. 3 shows the observed changes in the state of the ovaries of mature individuals taken during 1951–52 in Beaumaris Bay. The records are arranged to show the full annual cycle of ovarian development, which may conveniently be divided into three periods. These are demarcated on the graph by the letters A, B, C and D.

The ovary which will give rise to the subsequent year's brood begins to develop whilst the mature ovary or the fertilized eggs are still present. Its rudiments are first clearly discernible in mid-February (A) as a diffuse fatty tissue containing minute ova. During March and April the ovary grows rapidly, and by the end of May (B) this new ovary is almost full size. During this same period the ova are swelling rapidly as indicated by measurements of the mean size of ova throughout the population. The size of the ova continues to increase, though more slowly, throughout the remainder of the year, until they are of mature size and age in early February (C) and remain so until fertilized (C–D). As there are individuals with varying stages of ovarian

TABLE III. SIZES OF OVA AND FERTILIZED EGGS IN BARNACLES

(All figures are means of measurements on eggs from several individuals. The measurements listed in the first three columns of figures are in μ.)

Species	Diameter of ova when fully developed $(2r)$	Length of fertilized egg $(2a)$	Breadth of fertilized egg $(2b)$	Ratio of volume: ab^2/r^3	Ratio of length to breadth of fertilized egg: b/a	Volume of fertilized egg in ml. $\times 10^{-6}$
Balanus hameri	265	385	205	0·87	0·53	8·50
B. porcatus	225	307	168	0·76	0·55	4·52
B. balanoides	210	284	154	0·73	0·54	3·53
B. crenatus	170	237	120	0·69	0·51	1·78
B. perforatus	160	221	115	0·72	0·52	1·54
B. improvisus	123	161	93	0·75	0·58	0·73
B. amphitrite var. *denticulata*	120	150	90	0·71	0·60	0·64
Elminius modestus	125	190	93	0·84	0·49	0·86
Chthamalus stellatus	130	191	94	0·77	0·49	0·88
Verruca stroemia	140	205	100	0·75	0·49	1·07

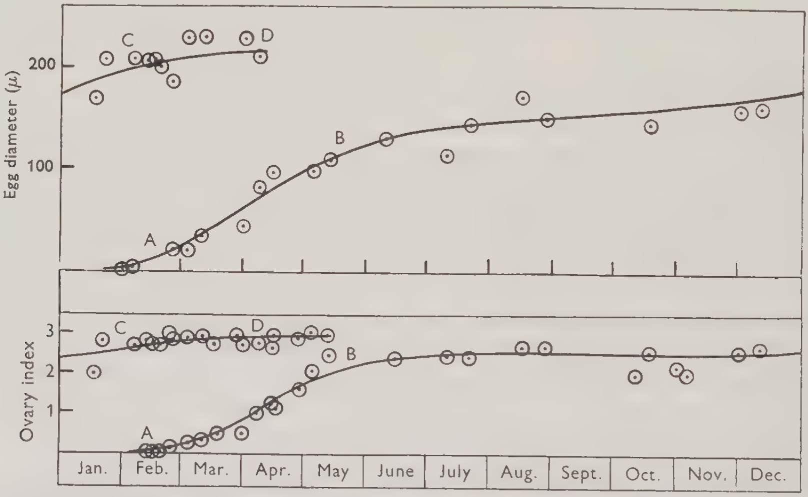

Fig. 3. Seasonal changes in the ovary. The upper curve illustrates the growth in size of ova, the lower curve variations in the ovarian index based on gross appearance (p. 479).

development included in both the curve of the growth of the ovary and that of the growth of the eggs, the slope does not exactly represent the rate of development in a single individual; this probably takes place more rapidly than is indicated by the curves.

The eggs become fertilized and discharged into the mantle cavity immediately after copulation during February. Fig. 4 shows the progress of fertilization throughout a population during 1952 (upper rectangle), together with offshore and inshore temperature records (lower rectangle).

The vertical lines associated with each point in the upper graph give the 20% confidence limits based on Stevens' tables. Thus the curve representing the progress of fertilization throughout the population should pass through at least four-fifths of these lines, and should be as close to the points as possible. The full curve illustrated refers to the numerous samples taken in Beaumaris Bay, and is satisfactory in not passing outside these limits. The onset of fertilization at Port Erin, represented approximately by the broken lines, occurred at about the same time, but the number of samples available was lower, and it was not possible to compare the exact dates very closely. It will be noted that the percentage fertilized rose to an average maximum of about 80% in the Bangor samples, but almost 100% at Port Erin. This was almost certainly a reflexion of the lower density of the population at Bangor. Despite their greater size their sparse distribution probably resulted in a proportion being too distant from their neighbours for copulation. The lower fertilization maximum is unlikely to be due to innate sterility, for the Bangor individuals showed greater growth and gonad development, and never harboured the isopod *Hemioniscus balani*, which sometimes exercises a sterilizing or debilitating effect.

It is interesting to note that if in place of percentages in Fig. 4 the probits (Finney, 1952) are plotted against time, there is a reasonable approximation to a linear relationship over the period during which fertilization is taking place (Fig. 4, inset). This indicates that the frequency with which fertilization occurs is normally distributed about a central point of time. At this point, when the probit is 5·0, 50% of the population bear fertilized egg-masses. This fact offers a useful means of obtaining the central or mean date of fertilization using all the available data. This is shown to be 17 February for the Beaumaris Bay samples at which time the temperature was at its minimum. From a similar procedure the central date of liberation of nauplii is found to be 1 April. The interval of 44 days is thus an estimate of the embryonic life in this area, based on all samples taken. The curve representing the liberation of nauplii in the Port Erin samples was again close to that of the Beaumaris Bay material.

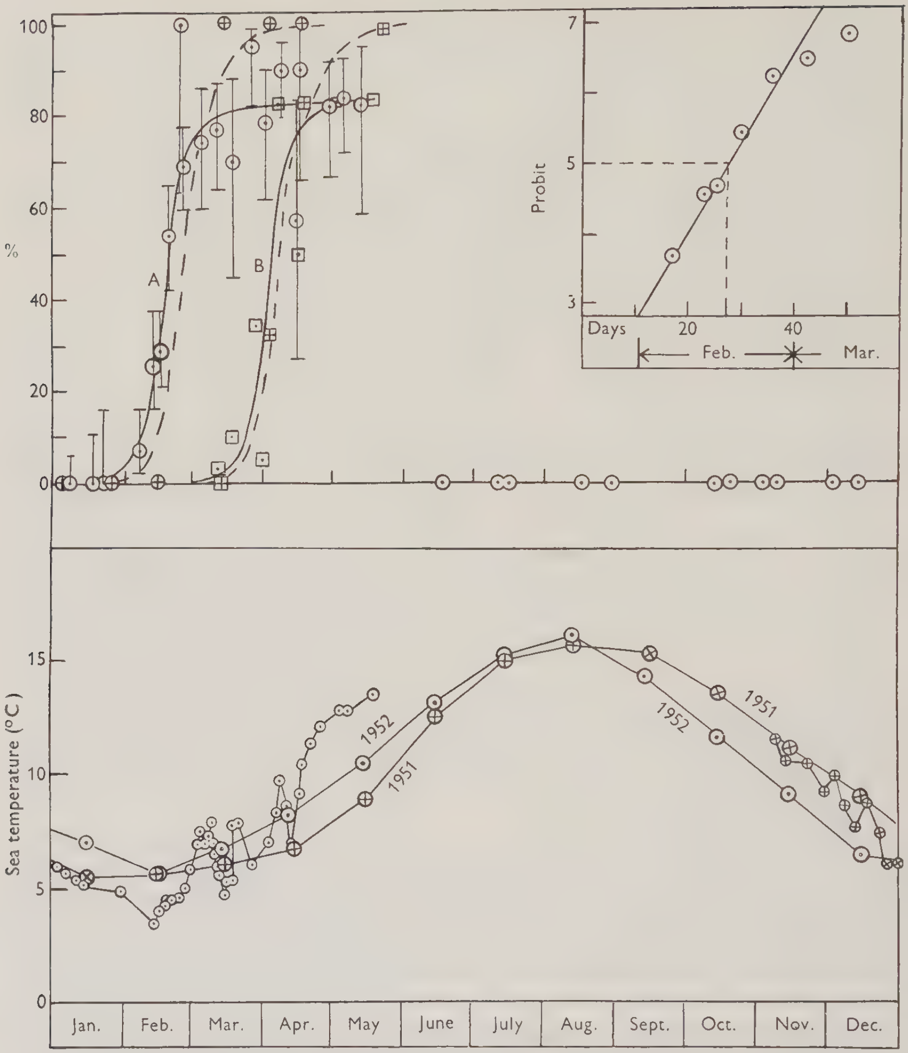

Fig. 4. Upper rectangle. Fertilization and liberation of nauplii in *Balanus porcatus* (1952). ⊙, percentage of individuals bearing or having borne fertilized egg masses in Beaumaris Bay samples; ⊕, ditto, Port Erin samples; ⊡, percentage of individuals having liberated their nauplii, Beaumaris samples; ⊞, ditto, Port Erin samples. To avoid confusion 20 % confidence limits are shown only for the points giving the fertilization of the population in Beaumaris Bay. Lower rectangle, temperature records for 1951 and 1952. ⊗, monthly mean offshore temperatures, Beaumaris Bay, 1951; ⊙, monthly mean offshore temperatures, Beaumaris Bay, 1952. ⊗, inshore temperatures, Menai Straits, 1951; ⊙, inshore temperatures, Menai Straits, 1952. *Inset.* Probit transformation applied to fertilization records in Beaumaris Bay samples.

Evidence for Obligatory Cross-fertilization

Cross-fertilization is known to be obligatory in *Balanus balanoides* (Chipperfield, unpublished thesis), in *B. crenatus* and in *Elminius modestus* (Crisp, 1950). The present samples offered suitable material to investigate *Balanus porcatus*, since the individuals were often found singly on bivalve *Modiolus* shells. Such individuals, which were termed 'isolated', sometimes constituted more than half the catch. Where two or more individuals were found on a shell these were termed 'adjacent'. This division was rigidly adhered to even if the two individuals were at the extreme ends or on opposite valves of *Modiolus* and appeared to have little opportunity of inseminating each other, or if a minute and apparently sterile individual occurred on the same shell as an otherwise isolated specimen. In both such cases the barnacles would be termed 'adjacent', since it was considered preferable to adhere to a definite criterion, rather than to attempt to make a judgement on the likelihood of cross-fertilization being possible, with a risk of introducing personal bias.

Table IV. Differences in Breeding Behaviour between adjacent and isolated Individuals

	Numbers with fertilized egg masses		Numbers with full size ovaries	
Date of sample	Adjacent	Isolated	Adjacent	Isolated
6. ii. 52	3/42	0/12	28/42	10/12
12. ii. 52	9/35	1/16	—	—
15. ii. 52	16/55	0/26	23/55	19/26
19. ii. 52	19/35	3/32	—	—
25. ii. 52	5/5	0/1	—	—
4. iii. 52	23/31	5/38	5/31	16/38
11. iii. 52	22/30	4/30	5/30	16/30
18. iii. 52	6/12	1/22	3/12	15/22
26. iii. 52	12/20	0/1	1/20	1/1
1. iv. 52	14/19	1/18	3/19	14/18
8. iv. 52	3/41	1/38	8/41	21/38
15. iv. 52	1/7	0/17	1/7	10/17
16. iv. 52	—	—	1/10	8/12
29. iv. 52	—	—	1/22	5/15
5. v. 52	—	—	11/32	3/4
13. v. 52	—	—	6/11	3/6
Total	133/332	16/251	96/332	141/239

Table IV gives a series of parallel counts over the breeding period on samples which contained 'adjacent' and 'isolated' individuals. It will be seen that only a small fraction of the 'isolated' samples ever contained fertilized egg-masses, yet the adjacent samples showed a maximum of about 80% bearing them. Similarly, the fraction containing full-size ovaries fell to a minimum in the 'adjacent' samples, whereas the proportion of isolated individuals with large ovaries was maintained without significant change, since none had discharged egg-masses. These differences over the whole set of samples are overwhelmingly significant. It is likely that before being dredged, a small number classed as isolated, might well have been in close proximity to individuals of the same species on other shells or objects nearby, whilst some of those classed as adjacent admittedly may have had little chance of insemination. The observed difference in behaviour of the two sets of samples is therefore slightly less than would be expected had an absolute division been possible into two classes, the one with and the other without mutual access for copulation. It is therefore clear that fertilization is at least greatly facilitated by individuals lying close together, and it is probable that this species also is an obligatory cross-fertilizing hermaphrodite. There is no indication that fertilization is merely delayed in isolated individuals, as would be expected if facultative self-fertilization were possible, and as apparently occurs in *Chthamalus stellatus* (unpublished data).

This study of the breeding cycle was therefore confined to adjacent individuals, except where otherwise stated.

Development of Embryos and Release of Nauplii

The fertilized eggs develop rapidly and uniformly into embryos, which remain separately enclosed in egg-cases retained in the mantle cavity of the adult up to the time of their liberation as stage 1 nauplii.

The egg-cases are transparent, and details of development may be discerned simply by teasing one of the paired masses of eggs on a slide and examining under low power. After fixation in 5% formaldehyde in sea water the eggs retain their typical appearance, but most other fixatives render them opaque or cause cytolysis. Apart from small variations during the earliest stages, the development of all the eggs present in a given individual is uniform.

The development of the embryos was referred to a scheme of clearly distinguishable stages as outlined in Table V. Groom's (1894) description of the development of the eggs of *Balanus perforatus* has been found to be applicable to this and to other species of barnacle; reference is therefore included in the table to the appropriate illustrations in Groom's paper.

Unfortunately it has not been found possible to promote development *in vitro*; hence the rate of development has been ascertained only indirectly by means of population studies. In a population where the onset of fertilization is gradual, a range of several stages of embryonic development is to be found in different individuals at any one time. If enough samples of *equal* size are examined at *regular* intervals, throughout the whole period during which egg-masses are present, the percentage of the total number of individuals which are in a given stage will clearly be proportional to the time which this stage occupies in the whole developmental period. If sampling is irregular suitable adjustments for the variation in numbers of individuals in the samples and for the different periods of time between taking samples are necessary. The proportionate time occupied by a given stage in the full term of development will then be given by the relation

$$t_n/T = \sum_i (S_n/S)_i \, \Delta t_i / \sum_n \sum_i (S_n/S)_i \, \Delta t_i,$$

where t_n is the time interval between stage n and $n+1$, $(S_n/S)_i$ is the fraction of the total number in the ith sample lying between stages n and $n+1$, and Δt_i is half the time interval between the $(i-1)$th and the $(i+1)$th sample.

TABLE V. THE DEVELOPMENTAL STAGES OF EMBRYOS IN THEIR EGG-CASES

		Corresponding stage from Groom (1894)			
			Figures illustrating stage in		
Stage	Description of development of embryo	Stage	*Lepas anatifera*	*Balanus perforatus*	*Chthamalus stellatus*
1	Unsegmented egg in oval or pyriform case	A	Pl. 14, figs. 1–7	Pl. 16, fig. 49	Pl. 18, figs. 83–86
2	Two simple blastomeres	B	Figs. 8–11	Fig. 50	Figs. 87–88
3	Upper blastomere divided, yolk not completely covered. 3 to 32 blastomeres		Figs. 12–19	Fig. 51; Pl. 17, fig. 64	Figs. 89 93
4	Yolk all undivided, completely or nearly completely covered by blastoderm cells		Pl. 15, figs. 20–23	Figs. 65–71	Fig. 94
5	Yolk cell divided in two by an oblique furrow, and completely covered by blastoderm cells	C	Figs. 24, 25	Fig. 72	Figs. 95, 96; Pl. 19, fig. 97
6	Yolk cell divided into 3 to 5 cells enclosed in blastoderm		Figs. 26, 27	Fig. 72*a*, *b*	—
7	Six or more yolk cells. Posterior thiceningk of mesoblast present		Fig. 28	Figs. 73–75	—
8	Embryo divided by two or more constrictions between rudimentary swellings giving rise to the appendages	D, E	Figs. 29–35	Fig. 76	—
9	Appendages clearly visible as short bifid swellings, setae absent or not evident	F	Figs. 36–37	Fig. 77; Pl. 18, fig. 80	—
10	Appendages with distinct setae. No eye visible	G	Figs. 38–40	Fig. 81	—
11	Median eye red or poorly pigmented, mass of yolk cells present	H	Fig. 41	Fig. 82	—
12	Eyes darkly pigmented, black or reddish brown; endoderm forms a clearly defined gut. Not hatching within a few minutes of placing in sea water		—	—	—
13	As 12, but more strongly pigmented. Hatching within a few minutes of placing in sea water. In formalized material a few often found freed from egg-cases		Fig. 42	—	—

Since T, the total time of development, is known, the absolute intervals t_n between the various stages can be computed. In this way a developmental time-table can be compiled (Table VI). It will be seen that the earliest developmental stages are quickly over, but that at the end of development apparently fully developed embryos (Stage 13) are retained for some time before being liberated. This results in a wide variety of embryonic stages being present simultaneously in the early part of development, and giving place to an appearance of greater uniformity towards the end. The degree of overlapping of embryonic stages is, however, limited, since each fertile individual produces one, but not more than one, annual brood.

During embryonic development the egg-masses change slowly in texture and appearance. When first discharged they appear bright creamy yellow and of the consistency of weak jelly, but gradually the egg-cases harden and the egg-masses become firmer. As the embryos develop more pigment, the colour of the egg-masses changes from yellow to ochre, then to fawn, and finally to dark brown. Each paired egg-mass is surrounded by an enveloping membrane in addition to the separate cases around each embryo. Immediately after fertilization spermatozoa may sometimes be seen between the fertilized eggs, but not within the substance of this enveloping membrane. Shortly before liberation which, like copulation, appears to be accompanied by a moult of the adult barnacle, the egg-masses soften and break down, so that the egg-cases lie almost loose in the mantle cavity. Hatching occurs after liberation into the sea. The hatching movements of the embryos are inhibited in water of low oxygen tension. Possibly the oxygen tension in the mantle cavity of the adult is lowered by respiration, so that hatching is prevented until the eggs are individually expelled from the mantle cavity, and exposed to a greatly increased oxygen tension in the surrounding water.

TABLE VI. DURATION OF EMBRYONIC STAGES IN *BALANUS PORCATUS*

Stage	Time of passing (days)	Duration (days)	Percentage of total time	Cumulative percentage
1	1·1	1·1	2·5	2·5
2	1·9	0·8	1·7	4·2
3	3·2	1·3	2·9	7·1
4	4·2	1·0	2·2	9·3
5	6·2	2·0	4·6	13·9
6	8·1	1·9	4·2	18·1
7	11·1	3·0	6·9	25·0
8	14·3	3·2	7·3	32·3
9	19·5	5·2	11·8	44·1
10	21·8	2·3	5·2	49·3
11	24·0	2·2	4·9	54·2
12	31·3	7·3	16·7	70·9
13	44·0	12·8	29·0	100

Liberation of first-stage nauplii begins towards the end of March and continues through the early part of April. They moult almost at once, giving rise to second-stage nauplii. These are normally the earliest stage found in the plankton and may be distinguished from the second-stage nauplii of *B. balanoides* by their slightly larger size and longer forked process. The remaining stages are also larger than those of *B. balanoides*, with which they might otherwise readily be confused. There is no evidence that nauplii retained in the mantle cavity developed further as suggested by Barnes (1953*a*); in one instance abortive egg-masses were found still remaining towards the end of April, but, unlike those reported by Barnes (1953*b*), these did not appear to be viable. Barnes (private communication) now considers that the larvae are invariably planktotrophic (Crisp & Knight-Jones, 1953).

The larvae have been found only in very small numbers in the plankton from Beaumaris Bay, but are much more common in samples taken in April and early May south of the Isle of Man, where the beds of *B. porcatus* are probably more extensive.

SETTLEMENT AND GROWTH

Not only have the larval stages been sparse in the Bangor area, but the settlement of the past two years has been correspondingly scanty compared with the heavy settlements of intertidal barnacles commonly observed. The proximity of the species to its geographical limits may explain its inability to produce heavy annual settlements as was also found in *B. balanoides* at Plymouth (Harris, 1946). Owing to the small numbers of spat found, it has not been possible to date settlement with great precision, but young spat have not been observed prior to the first week in May, and the main settlement appears to take place in the latter half of this month. By July considerable growth has occurred, and by August it is not easy to distinguish the settlement of the current year from the smaller individuals of the previous season. This difficulty results from the considerable individual variation in rates of growth, a feature which appears to be common in cirripedes, and is probably largely due to environmental differences.

Fig. 5 shows in histogram form the size-distribution of three samples of *B. porcatus*, the first taken in mid-April, a month before settlement; the second at the end of July, about 2½ months after settlement; and the third in December. The absence of well marked peaks indicating year-groups is due partly to variation in growth rate and partly to the small spatfalls of 1951 and 1952, which were scarcely sufficient to maintain the population at its present level and constituted only a small fraction of the total.

There can often be noted on the parietes of the shell of an old individual a series of slight concentric irregularities or ridges (Fig. 6), at which the angle of the shell changes slightly but abruptly. These discontinuities are not directly associated with moults, for animals kept in shallow dishes in the laboratory moult at frequent intervals; whereas no more than four such shell discontinuities are discernible on most large individuals. The histograms shown in Fig. 7 illustrate that the discontinuities measured from the apex of the barnacle form a regular series of frequency peaks. Slight changes in the thickness of the basis can also be observed, usually, but not always, agreeing in number with the discontinuities of the shell. It seems probable that both the parietal ridges and the basal growth bands are due to annual changes in the rate of growth. By equating the number of growth bands to the probable age, the individuals from which the histograms shown in Fig. 5 were derived were grouped, and the results of this grouping are shown as divisions within

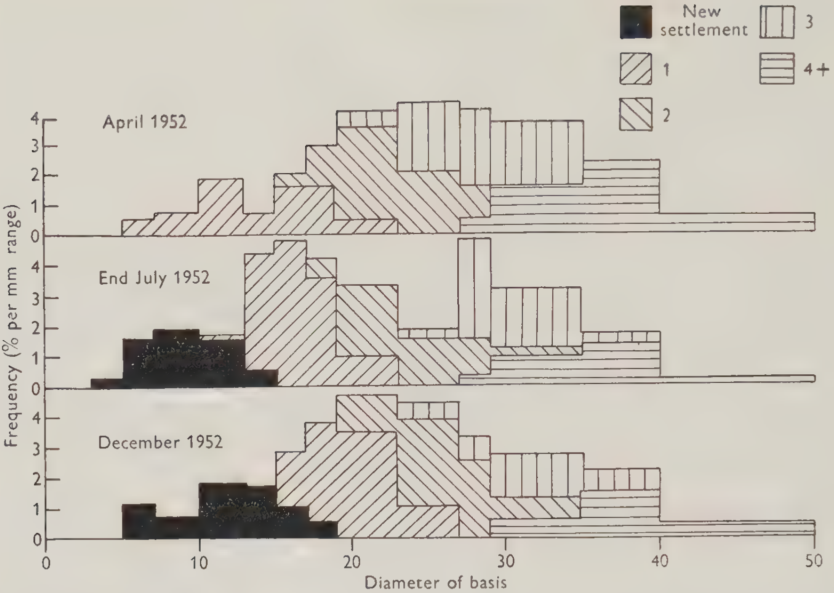

Fig. 5. Histograms of size frequency in *B. porcatus*. The size is measured as mean basal diameter in millimetres. The histogram is divided internally to show year groups, 1 being those in their first year of growth in April 1952, 4+ being those in their fourth or later years.

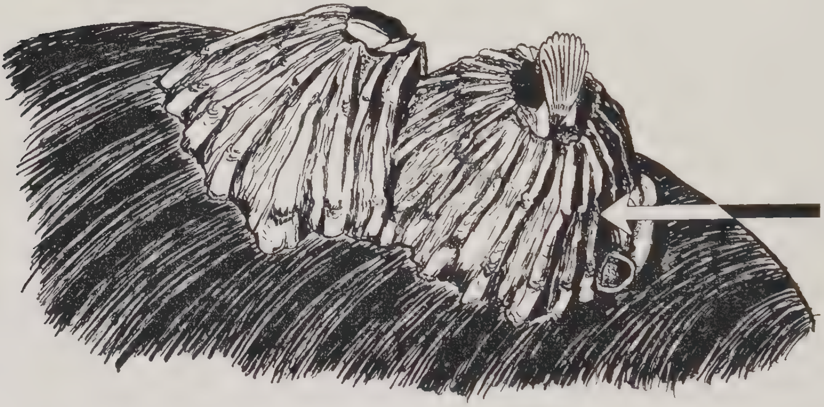

Fig. 6. Two individuals of *B. porcatus* in their third year of growth. The arrow shows in the right hand individual the position of the rather prominent parietal growth ridges corresponding to the first year; the other growth ridges are present but are only just discernible in the original.

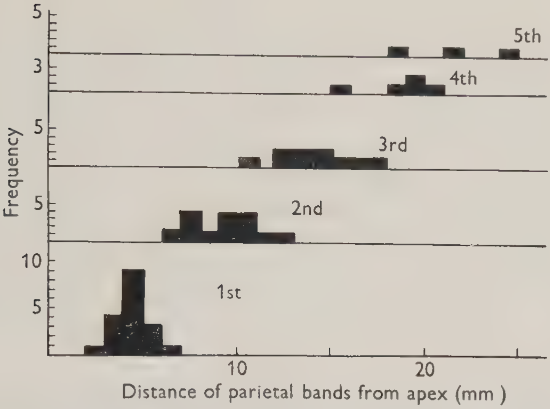

Fig. 7. Histograms relating to *B. porcatus* selected because they showed growth bands on the parietes. The frequency of occurrence of bands is plotted against the distance measured vertically (in mm) from band to apex. The horizontal scale therefore represents the probable height of the barnacle when the growth ring was formed, if increase in height is due solely to basal growth.

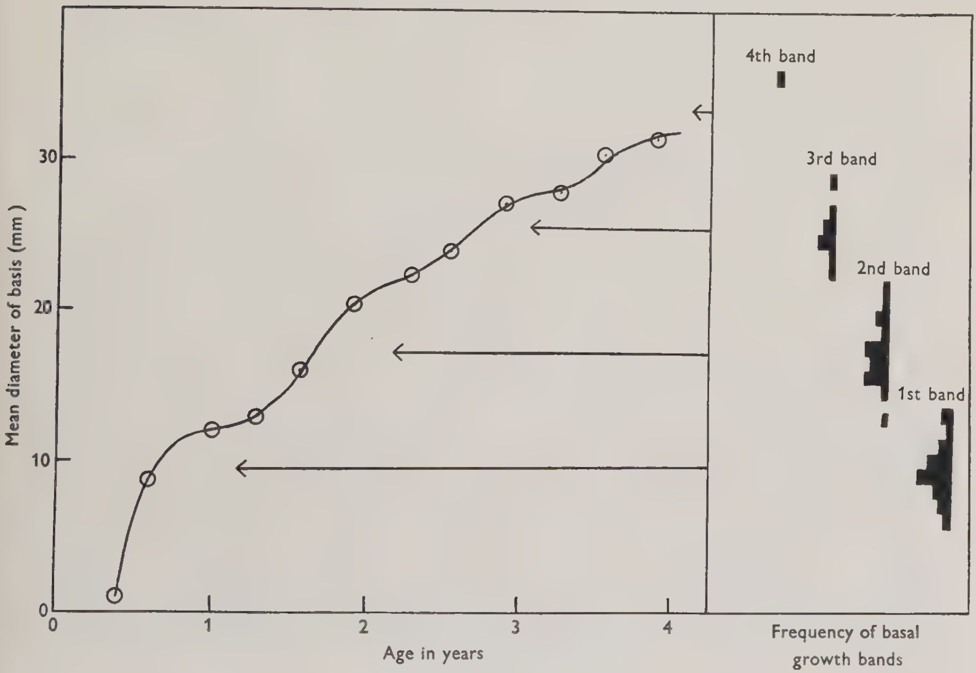

Fig. 8. Growth rate of *B. porcatus* in Beaumaris Bay. The horizontal axis gives the estimated age, the vertical axis the mean basal diameter from the histograms illustrated in Fig. 5. On the right-hand side are shown histograms of the basal growth marks of a few individuals in which they were clearly distinguishable; these correspond approximately to annual rings.

the histogram. From the mean size of each of these age-groups, a curve showing the mean growth rate of the population has been derived (Fig. 8). To check the validity of the method the mean sizes of the growth bands in the bases of a number of well-formed individuals were measured, and histograms based on measurements of the 1st, 2nd, 3rd and 4th bands are inserted on the right-hand side of the graph. The mean diameter of each growth band agrees reasonably well with the mean population size of each year-group. Neither the parietal ridges nor the basal growth bands seemed generally to be sufficiently reliable guides to age to enable estimates to be extended beyond the fourth year of growth. The largest individuals in the population, of between 40 and 50 mm basal diameter and 20–30 mm height, were probably some 5 or 6 years old.

Individual shape varies considerably according to the substratum on which the barnacle grows. In general, growth on the convex part of a *Modiolus* shell tends to give a tall individual, particularly if the edge of the barnacle reaches the edge of the molluscan shell and cannot grow further. Conversely, *Balanus porcatus* growing on a concave surface, as for example on the inside of a dead *Modiolus* shell, is often flatter than usual. There is also a marked tendency for the rostral plates to spread over the substratum more than the carinal. Thus the carinal plates approximate more closely to the perpendicular and may be slightly concave (Fig. 6), while the rostral plates are more sloping and convex, and cover a greater area. A greater area is thus available for attachment and growth of new individuals at the rostral end, which may partly account for the observation by Barnes (1953*a*) that young barnacles occur in greater numbers on the rostral than on the carinal side of the aperture. As growth proceeds the ratio of height to basal diameter increases slightly; this increase is particularly marked in older barnacles which have grown against the edge of the shell or against other barnacles, when upward growth tends to replace lateral. Fig. 9 shows the mean ratio height/basal diameter plotted against basal diameter for various age-groups, based on individuals collected in July 1952. Inserted in this graph are values of the ratio height/diameter based on the mean measurements of the heights of the parietal and diameters of the basal growth bands; these show fair agreement with the ratios observed directly. The correlation between the dimensions obtained from marks on the parietes and those on the basis further suggests that they represent growth-rings, and indicates also that *Balanus porcatus*, like *B. balanoides*, grows mainly from the basal perimeter. Moreover, if this interpretation is correct, the degree of erosion at the opening of the barnacle appears to be small.

DEVELOPMENT OF GONADS IN RELATION TO AGE

Fig. 10 illustrates the relation between size and gonad development, scored as described above (pp. 475–9). The data on which this graph is based are necessarily restricted to those seasons when the gonad is normally well developed in

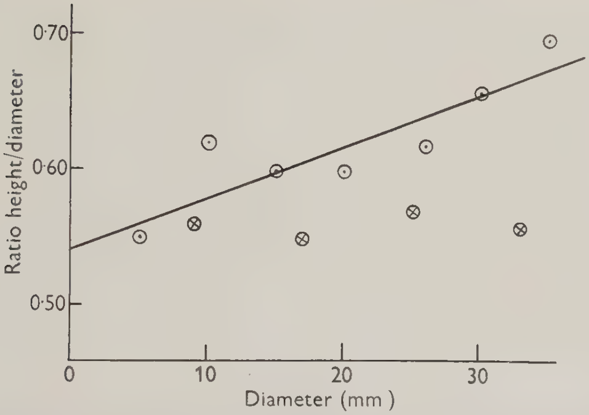

Fig. 9. Ratio of height to basal diameter in *B. porcatus*. ⊙, mean ratios based on measurements of groups of individuals of a given size range; ⊗, ratios based on mean values for 1st, 2nd, 3rd and 4th parietal and basal growth marks respectively.

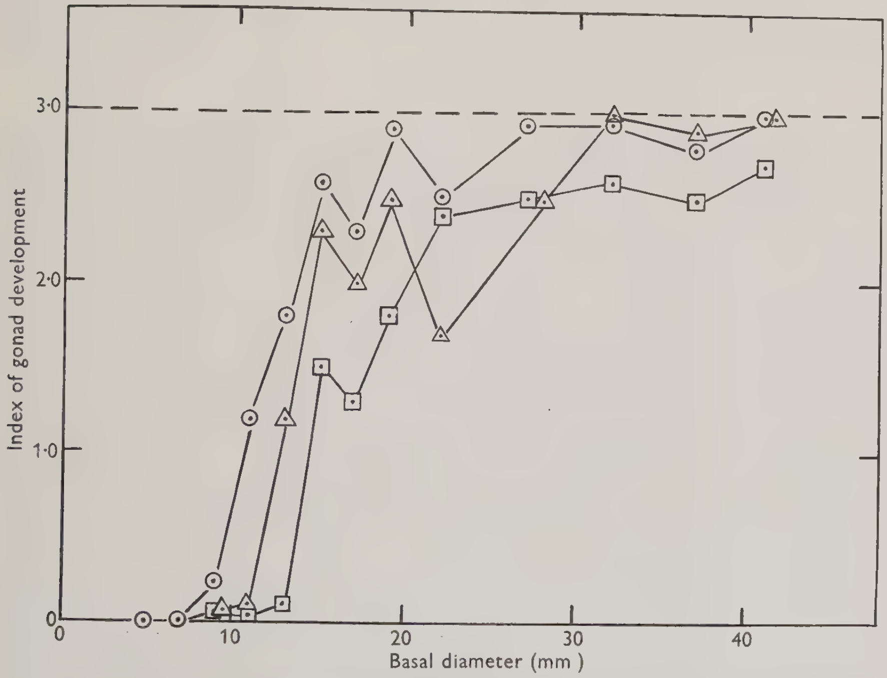

Fig. 10. Growth of gonads in relation to mean basal diameter (mm). ⊙, Index of development of ovary (p. 479). △, Index of development of vesiculae seminales (p. 475). ⊡, Index of development of testis (p. 475).

mature barnacles. Comparing this graph with that of Fig. 8, it will be seen that barnacles in their first year have just attained an average size consistent with breeding (13 mm). The range of variation is such, however, that a percentage of this group, particularly those of less than 9–10 mm in diameter, often fail to breed in their first year. By the second year and thereafter however, full maturity is attained and all individuals, unless isolated or parasitized, breed normally. No evidence was found for senility among the later age-groups as described by Moore (1935) in *B. balanoides*.

It will be seen from Figs. 1 and 10 that accelerated growth of the penis and initial growth of the ovary, testis and vesiculae seminales take place almost simultaneously, namely during the time that the mean diameter of the barnacle increases from 6 to 14 mm. This corresponds to the latter part of the first year of growth (Fig. 8) at a time when the water temperature and assimilative growth rate are low. There is no evidence at present to suggest that any one of the reproductive organs precede in development or initiate the growth of the others. The slightly earlier recorded appearance of the penis and ovary may be due only to the greater facility with which these structures can be observed in a rudimentary state, for the testes also can often be distinguished in individuals of less than 9–10 mm diameter if a sufficiently careful examination is made.

Comparison with other British Barnacles

Both in habitat and breeding behaviour *B. porcatus* most nearly resembles *B. hameri*, which in the Irish Sea copulates during January, and hence liberates its nauplii and settles a little earlier than *B. porcatus*. Both are large species, having an annual reproductive cycle, and therefore only one generation of larvae each year.

The number of larvae in each brood is correspondingly large; thus a specimen of *B. hameri* of 24 mm diameter, 25 mm height, and about 20 g weight, contained approximately 100,000 embryos, whilst a large specimen of *B. porcatus* of 35 mm diameter, 15 mm height, and 13 g weight, contained approximately 50,000. In both species the egg-masses together accounted for 8–10% of the total wet weight (including shell) and about 35% of the wet weight of the soft parts only. In smaller individuals the weight of the naupliar masses compared with the weight of the barnacle is less and the number of embryos far fewer. A similar relation was noted by Moore (1935) in *B. balanoides*, where 5000 to 10,000 embryos were produced by fully grown barnacles, but only a few hundred in those which were mature in their first year. *B. balanoides* is also the only other British species known with certainty to produce only a single annual brood.

B. porcatus, *B. hameri* and *B. balanoides* are all boreal-arctic forms reaching their southern limits in British waters. All three species therefore accord with the principle, enunciated by Appellöf (1912) and Orton (1919), that animals living in the warmest parts of their geographical range breed in the coldest months of the year. Thus the approximate periods during which embryos are found in the mantle cavity in British waters are: *B. balanoides*, November–March (Moore, 1935); *B. hameri*, early January–early March (unpublished data); *B. porcatus*, February–March.

Orton (1919) quotes, in support of this general principle, *Antennularia ramosa* and *Bougainvillia ramosa* as being winter breeders in the Mediterranean, and early-summer breeders at Plymouth, pointing out that well-defined temperature limits control the period of breeding of such species and account for their latitudinal variations in the times of breeding. It seems unlikely, however, that boreal-arctic barnacles breed during the warmer months even in high latitudes. In *Balanus balanoides*, in particular, it is known that at higher latitudes the onset of breeding occurs earlier, but only sufficiently so to compensate for more prolonged development during the colder winter. The embryos are therefore still retained throughout the coldest months, although copulation occurs slightly earlier in the autumn and liberation slightly later in the spring.

It seems probable that in these species the annual cycle is so adjusted that over a wide range of latitude their larvae are liberated in the spring, when abundant phytoplankton is available, and when conditions are favourable for rapid and prolonged growth after settlement. The rapid ovarian development which occurs during early summer in all these species, but which does not lead immediately to reproduction, may be primarily an adaptation to arctic conditions when the growing season is short. Reproduction is postponed until winter, so that the planktonic stages will not be thrown on their own resources until the spring, when the adults too begin to assimilate reserves for the following winter's breeding cycle. This behaviour is in marked contrast to that of warm temperate barnacles such as *Elminius modestus*, *Chthamalus stellatus* and *Balanus improvisus*, which produce several broods during the summer months, but lie dormant and without obvious food reserves during the winter.

The marked recession of the testes and vesiculae seminales and the slight decrease in length of the penis, that occur in *B. porcatus* immediately after fertilization, occur also in *B. hameri* and are very pronounced in *B. balanoides*, where the male organs regenerate rapidly only a few months prior to copulation (Chipperfield, unpublished). Both in *B. balanoides* and *B. porcatus* it has been shown that this recession is seasonal and occurs whether or not copulation and discharge of eggs by the ovary have taken place. It is therefore improbable that the growth of the male gonad is controlled by any hormonal influence from the ovary, as might have been thought possible from the time relations of the development of these organs.

The onset of copulation corresponds to the coldest part of the year (Fig. 4), but it is not known how far it is directly controlled by temperature. In deep turbid water temperature is probably the only reliable indicator of season; salinity, light and biological factors varying widely from year to year. A simple relation between the onset of breeding and the immediate temperature seems, however, rather unlikely, for there is a considerable variation in the minimum temperature reached, not only year by year but also at different depths, and this would make it very difficult to explain the regularity and uniformity in breeding shown in different seasons. A direct stimulus due to change of temperature is also improbable in this species since breeding commences when the temperature changes are minimal. It is considered more likely that the gradual fall in temperature throughout autumn and winter sets in motion metabolic changes causing gradual ripening of the gonads. If these changes were suitably geared to the falling temperature gradient, copulation could follow as a result of the maturation of individuals at the appropriate season without an immediate external stimulus. The normality of the distribution of fertilization times (p. 481) is probably a reflexion of a normal distribution in the development of the gonads, and therefore supports this view. Were an external stimulus responsible, one would expect a sudden onset of fertilization in which all the ripe individuals would take part, followed by a gradual tailing off as those developing later became mature.

The growth rates and longevities of *B. porcatus* and *B. hameri* are similar; their attainment of maximum size is very gradual, but mortality is much lighter than in the smaller species of barnacles. The thick shells of *B. porcatus* do not rapidly decay, yet relatively few dead ones are found among the early year-classes. This is probably related to the quieter conditions obtaining off-shore. It is somewhat surprising, in view of the remarkable similarity in the ecology of these two large species, that their general shapes are entirely different, and that the shell of *B. porcatus* is extremely thick and the compartments well knit together, whereas that of *B. hameri* is rather fragile.

My thanks are due to Prof. F. W. Rogers Brambell, F.R.S., for his continued hospitality during the period that the Marine Station was still unready for occupation, to Dr J. S. Colman for arranging the collection and sending of numerous samples of *B. porcatus* from the grounds near Port Erin, and to Dr E. W. Knight-Jones, who made a number of collections on my behalf before my arrival in Bangor, and who kindly read the text and suggested a number of improvements.

SUMMARY

Balanus porcatus is a cross-fertilizing hermaphrodite which breeds once a year, producing some 50,000 embryos in each brood.

In the Irish Sea copulation and discharge of eggs into the mantle cavity takes place in February and liberation of nauplii in March. During copulation

spermatozoa are injected into the mantle cavity and ova are discharged simultaneously. The spermatozoa become enclosed with each mass of eggs in an enveloping membrane which softens only when the embryos are fully developed and ready to hatch. Hatching occurs after liberation into sea water and is probably induced by the higher external oxygen tension. After a larval development lasting 6–8 weeks in the plankton, the cyprids settle in May and June.

In mature individuals the testes develop and the vesiculae seminales gradually fill during summer and autumn. The penis, though present throughout the year, becomes somewhat enlarged in mid-winter just before copulation. Prior to copulation the testes begin to degenerate, and just after copulation the vesiculae seminales shrivel and the penis becomes somewhat shortened.

The ovaries re-develop during spring and summer, the bulk of ovarian tissue being assimilated by autumn. The ova, however, increase in size steadily throughout the year, attaining maximum size and ripeness by mid-winter.

The rate of growth has been measured by noting the numbers of growth marks on the parietal plates and bases of the shell. The parietal marks are more distinct and are due to the angle of growth of the shell varying slightly during each season. Individuals commonly live for 3 or 4 years reaching a basal diameter of 30–40 mm: some probably survive for as long as 5 or 6 years. Some mature in their first year. Both male and female gonads appear when a basal diameter of about 10 mm has been attained, and the penis simultaneously undergoes rapid heterogonic growth.

It is suggested that the winter breeding of this species, of the closely similar *B. hameri*, and of *B. balanoides*, is an adaptation enabling the nauplii to be liberated as early as possible in the spring. They thus have the maximum available time to establish themselves during the short summer of higher latitudes. The main period of assimilation of the adult also falls during spring and summer, a long period with full gonads intervening before the postponed onset of breeding.

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J. Mar. biol. Ass. U.K. (1954) 33, 497–509
Printed in Great Britain

THE SUSPENDED MATTER IN SEA WATER AND ITS SEASONAL CHANGES AS AFFECTING THE VISUAL RANGE OF THE SECCHI DISC

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(Text-figs. 1 and 2)

In a previous paper (Armstrong & Atkins, 1950), 20 l. samples from International Hydrographic Station England No. 1 (E 1), about 10 miles south-west of the Eddystone in the English Channel, were filtered through collodion membranes of 1·09μ average pore diameter (A.P.D.).

Between June 1948 and November 1949 the residue from these surface samples, when dried and ignited, amounted to 0·45–2·77 g/m^3 (or parts per million). These deposits consisted of 55–17 % silica, 28–3 % ferric oxide, 20 to under 1 % of alumina, and 70 (or, excluding one high value, 29) to 9 % calcium carbonate. A few determinations of insoluble organic matter gave to 1·15–1·77 p.p.m. when dried at 100° C. Pettersson (1934*b*), using the glass filters of Schott (Jena), found 1·4 p.p.m. for the total organic and inorganic matter similarly dried, from surface water in the Gullmar Fjord during the spring outburst.

The suspended matter, organic, and inorganic, is responsible for by far the greater part of the scattering of light (Atkins & Poole, 1952), the extinction of which is due to absorption by the water and suspended matter and to scattering.

The difference in the optical properties of water samples depends upon the amount and properties of the suspended matter and the 'yellow substance', which is supposed to be in solution. Furthermore, as the scattering produced by very fine particles affects the short wave end of the spectrum more than the rest of it, it acts in a manner similar to a yellow filter, and as far as we know 'yellow substance' has never been isolated. In the red and the deep red the much increased absorption reduces the relative importance of the scattered light.

The Comparison of Suspended Matter on Filter Discs

As shown by Atkins & Jenkins (1953) a good comparison can be made visually and in photographs; it is easy to place the discs in order of increasing deposit, when 1 l. or 2 l. of water are passed through a collodion membrane. Ordinarily the discs are of a greyish mud colour, tinged sometimes with faint yellowish green. On 21 October 1952, and on 25 March 1953, the deposit was a light buff darkening with quantity to chocolate. This happened again on 5 October 1953.

The discs were photographed to get a permanent record. This places them in relative order for each series, but it is well known to be very difficult to get an accurate measurement of intensity of grey from a photograph.

Accordingly we determined the diffuse reflexion relative to that of an unused disc, namely the relative albedo. For comparison we also determined this in the photograph for 21 January 1953, taking the white filter-paper background as 100. Actually its reflexion may be higher or lower than that of the filter disc, but, as shown in the print reproduced in Fig. 1, the two were in some places indistinguishable, or in others the filter disc was very slightly the lighter. The effect would be to give a relative albedo 1% or 2% too high.

The relative albedo was found as follows. A selenium rectifier cell was mounted in the place of an eye-piece on an old microscope stand, on the stage of which the sample discs were placed inside a ring immediately beneath the tube, which was devoid of lenses. The discs were illuminated using a 36 W 12 V car headlight, at about 45° angle of incidence. The cell was racked up till its field of view embraced the greater part of the disc. With this arrangement a dry collodion disc gave 160–200 scale divisions when the cell was connected to a galvanometer of sensitivity $0{\cdot}946 \times 10^{-9}$ A/mm and resistance 686 Ω. Even with such a high resistance the selenium cell showed a rectilinear relation between current and light for such small currents, under $0{\cdot}2\,\mu$A. It was found that, with the depths of tint given on the discs by 2 l. of water from station E1, the relation between quantity of suspended matter and tint was rectilinear. Thus the line for a sample as obtained and when diluted with distilled water was rectilinear for 75, 50, 25, 20, 15, 10, 5% sea water plotted against the albedo. The inorganic suspended matter was thus studied along with the phytoplankton.

It had been shown by Atkins & Poole (1952) that the scattering of light by surface samples of sea water was usually greater than that by the deeper water. To ascertain the variation with depth of the residue of the suspended matter, after extraction with 80% aqueous acetone to remove chlorophyll and carotenoids, the discs were ignited and weighed. The samples were taken at Station E1, 19 June 1952. The photograph of these discs before extraction is shown in Atkins & Jenkins (1953). The ignited residue was $1{\cdot}6$ g/m^3 for surface water; 1·0 for 5 and 10 m, and $0{\cdot}4$ g/m^3 for 15, 20, 25 and 50 m. It is clear that an estimate based upon surface samples only may be much too high. The change in amount of residue did not coincide with the thermocline. The aqueous acetone extraction probably left some sea salt behind, but its major constituent, sodium chloride, would have been volatilized during the ignition, so it is unlikely that sea salt introduced an error. Under the microscope the residue appeared to be mostly amorphous, but with numerous crystalline mineral particles.

Taking the above values for mineral suspended matter and interpolating suitably, the 0–20 m column for 19 June was found to contain 18·0 g, which may be taken as having sp.gr. 2·8, and so a volume 6·4 cm.^{3} If the suspended matter consisted of little cubes of 1μ sides, a limit that might not pass the collodion filter, there would be $6{\cdot}4 \times 10^{12}$ of them in a 20 m^3 column, a depth at which one might see a Secchi disc in clear water. The number given is equivalent to having 32 in a haemocytometer field, 1 mm^2 in area and 0·1 mm deep. Of this area the cubes would obstruct $32\mu^2$, out of the total $10^6\mu^2$. Thus were the cubes entirely opaque and—quite impossible of course—arranged so as not to overlap—one would have a complete screen cutting off all light in 3·12 m. It so happened that on this date, 19 June 1952, the Secchi disc could be seen to 23 m, the maximum for the years 1948 to 1953 inclusive. The date also gave a minimum for the chlorophyll for that summer, and with the assumptions made in the earlier paper the 37 mg of chlorophyll found in the 20 m column would indicate 6·4 g for the wet weight of the phytoplankton. Obviously this would give the same number of $1\mu^3$ organisms as there were mineral particles, and the size is a possible one. These, however, would be nearly transparent and the mineral particles would have a higher refractive index and would produce a greater effect optically. The coincidence in the numbers of particles is quite fortuitous, and occurred when the phytoplankton was at a minimum.

It is obvious that if the particles (or organisms) had sides 2μ in length, the volume would have been $8\mu^3$ with $0{\cdot}8 \times 10^{12}$ particles in a 20 m column, and only four, with area $16\mu^2$, in the volume of the haemocytometer field. Thus as the size increases the scattering and obscuration occasioned by the same mass of particles is much reduced. It follows that the optical effects produced by equal numbers of different species of the phytoplankton may be very different.

Microscopic Examination of the Suspended Matter

Dr A. G. Lowndes kindly examined some of the filter discs, which were handed to him after the plant pigments had been removed by 80% aqueous acetone in the cold. They were examined first of all by means of strong reflected light under a low-power binocular microscope, magnification 20 diameters, and presented a ground mass of varying colour which could not be resolved even under much higher powers. The ground mass appeared to be a fine mud or clay. A number of black specks and fibres were also present. These were removed, mounted in Canada balsam and observed with crossed nicols. The fibres were from wood and the remains of rope or twine. The wood fibres consisted of both oak and pine or woods closely allied to them. The other fibres consisted of such things as manila and coir and possibly a little jute. The black or dark specks consisted of carbonaceous matter, probably to be associated with soot, pitch or tar. These were readily volatilized when heated on the corner of the slide over a bunsen. Other dark specks consisted of oxides of iron, possibly from the stokeholds of ships. Fragments of shell were always present. There was very little actual mineral matter, other than the clay, except for an occasional grain of quartz or cordierite one of which was about 0·1 mm in diameter. There was also a small amount of kaolinite. There were occasional sponge spicules, also diatoms and copepods.

Some incinerated residue from the discs of 19 June was also examined. This consisted of the minerals quartz and cordierite, also some micaceous matter, probably sericite. Broken grains of felspar (orthoclase) were also present, as well as a small fragment of tourmaline. Most of these grains were under 40μ in diameter. Dark grains of iron oxides were fairly abundant.

Cordierite (syn. iolite) may be found in minute crystals in granite and is not very common. It is a silicate of the oxides of aluminium, magnesium and iron. Orthoclase felspar is typically a silicate of aluminium and potassium and is common in granite in Cornwall and in most granites everywhere. Tourmaline also is common in granite and varies in composition, it usually consists of silicates of aluminium, sodium and boron.

The Variation in Suspended Matter Throughout the Year

The greater part of the organic suspended matter arises from the phytoplankton and a quantitative study of this, based upon the chlorophyll extracted from the filter discs, was made for 1951–52 by Atkins & Jenkins (1953).[1] But the collodion discs do not appear to alter greatly in depth of colour when extracted with 80% acetone, so it seems that the greater part of the residue, organic and inorganic, remains on them. From June 1952 to January 1953 photographs of the discs were obtained. Those for June and July 1952 have already been published and may be compared with Fig. 1 which shows the discs for 22 September 1952 and 21 January 1953. In September the surface disc is the darkest, and Table I shows that this is quite usual, but it is not usual to have the other discs so like the surface; from 15 to 50 m the water seemed uniform, with 5 and 10 m slightly poorer in suspended matter. This uniformity is the result of the vertical mixing of the water, the whole column having just reached the isothermal state. Such a column is less stable than one with an upper warm layer, but in spite of this and the fact that the water had cooled from 14·24 to 12·31° C in the 8 weeks' interval, the November column gave a very dark surface disc, and, after some mixing in December, so did that of January, as shown in Fig. 1. With very little plankton at this season, the effect must be almost entirely due to lifeless matter, much of which rapidly re-accumulates near the surface. It is not known how thick this layer is, but it is remarkable that photoelectric measurements of submarine light intensity have often shown a surface loss for which a satisfactory explanation has been

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[1] In this paper the following *corrigenda* should be noted. At the bottom of p. 501 and top of p. 502, 'g.' (in three places) should read 'kg'. In the Summary (6th and 7th lines) 'wet weight of phytoplankton' should read 'chlorophyll'.

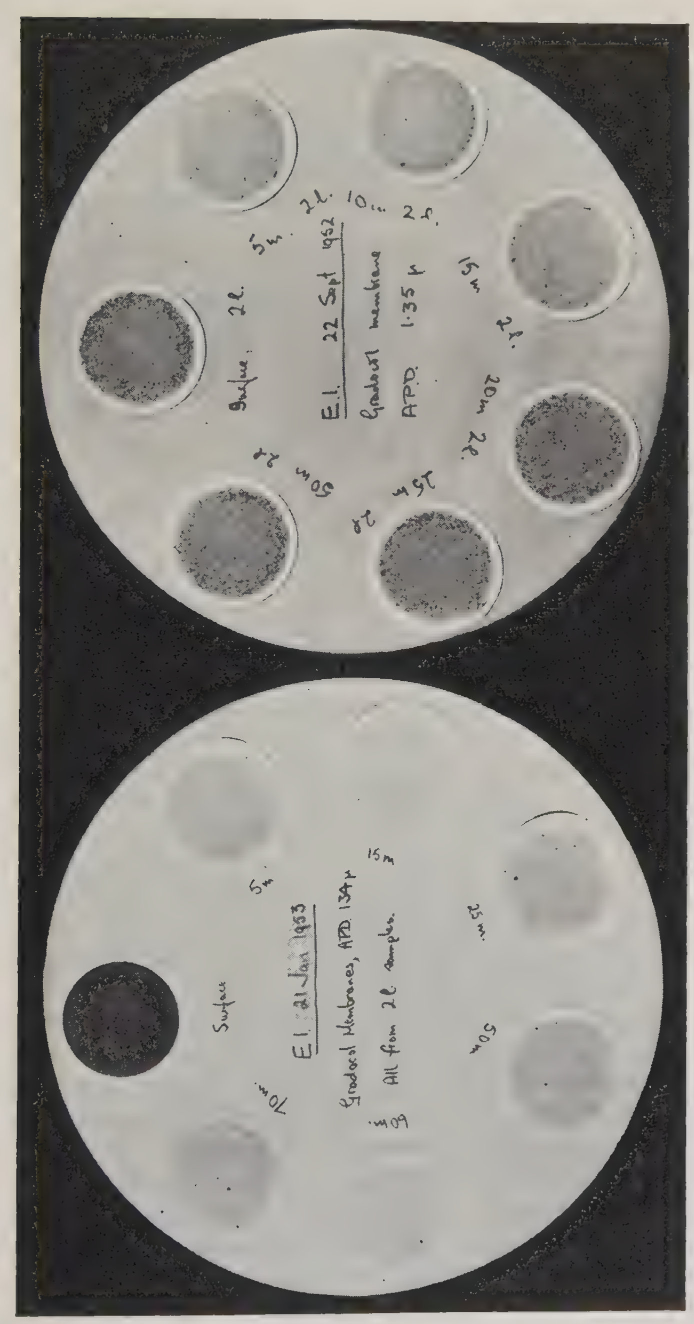
E.I. 22 Sept 1952
Gradocol membrane
APD. 1·35 μ
Surface, 2l.
5m. 2l.
10m 2l.
15m 2l.
20m 2l.
25m 2l
50m 2l
E.I. 21 Jan 1953
Gradocol Membranes, APD. 1·34 μ
All from 2l samples.
Surface
5m
15m
25m
50m
60m
70m

Fig. 1. Gradocol membranes after filtering 2 l. of water from Station E1 September 1952 and January 1953.

difficult. But when the suspended matter is much reduced the phytoplankton portion may cause a maximum at a considerable depth, 25 m, as on 15 July 1952, on 16 March and on 10 August 1953.

As previously explained, in order to get quantitative results, recourse was had to determinations of the relative albedo of the discs. Table II shows that the albedos of the photograph tend to increase the contrast somewhat. But the agreement of the visual examination and albedo methods can be very good as far as the placing in decreasing order is concerned. Thus for 16 March the two agree exactly save that the albedo places the 5 m as seventh and the 70 m as sixth, whereas the visual comparison reversed the order. Here, however, the albedo was 74 for 5 m and 73 for 70 m and errors larger than this could easily occur. On 10 August, however, with very pale discs, agreement was not at all as good, but five discs had albedos of 82–84%.

TABLE I. ORDER OF AMOUNT OF DEPOSIT ON COLLODION FILTER DISCS

The depths are placed in ordinal rank according to the amount of deposit on the collodion filter discs, the darkest colour ranking first, from June 1952 to November 1953. Two litres of water filtered through each from depths shown, at International Hydrographic Station, E1.

Depth (m)	19. vi.	15. vii.	20. viii.	22. ix.	20. xi.	22. xii.	21. i.	24. ii.	16. iii.
0	1	2	1	1	1	1	1	1	2
5	3	4	4	7	6	4	5	3	6
10	6	7	5	6	—	—	—	—	4
15	6	4	1	2	2	4	6	2	2
20	5	4	3	2	—	—	—	—	4
25	3	1	5	2	6	4	2	3	1
30	—	—	—	—	—	—	—	—	9
40	—	—	—	—	—	—	—	—	—
50	2	2	5	2	2	2	2	3	8
60	—	—	—	—	—	4	6	—	—
70	—	—	—	—	—	2	2	—	7

Depth (m)	25. iii.	27. iv.	22. vi.	20. vii.	10. viii.	8. ix.	5. x.	20. x.	17. xi.
0	5	1	1	7	5	7	2	1	1
5	2	5	2	2	5	1	9	2	2
10	7	—	—	—	4	3	1	6	4
15	6	5	5	7	8	5	8	3	3
20	7	8	4	5	2	6	4	5	5
25	9	8	3	6	1	9	5	4	5
30	10	5	5	7	3	4	7	7	9
40	2	2	—	4	—	—	—	—	—
50	2	3	5	2	8	2	6	7	7
60	—	—	—	—	—	—	—	—	—
70	1	3	5	1	7	8	3	7	8

Going back to the re-establishment of layering as indicated by the photographs for the November and January discs, there is evidence of long standing that this is due to the presence of plankton or to organic matter. It is shown by a study of the 'respirable carbon' (Atkins, 1922), for on storing water taken from E1 on 9 November 1921 and re-determining its pH value at intervals, it was seen that the production of carbon dioxide was markedly greater between 20 and 25 m than elsewhere, with a minor peak in the graph at 5 m as seen in the 1921 figure.

TABLE II. RELATIVE ALBEDO OF COLLODION DISCS DURING 1953

The discs had filtered 2 l. of sea water taken at various depths from Station E 1 during 1953. The second column shows the relative albedo of the photographic print of the January series, alongside of the direct test on the disc as in column 3.

Depth (m)	21. i.	21. i.	23. ii.	16. iii.	25. iii.*	27. iv.	22. vi.†	10. viii.	8. ix.	5. x.*	20. x.	17. xi.
0	*29*	25	64	66	73	55	37	84	73	49	60	58
5	*88*	68	81	74	64	66	49	83	54	61	64	64
10	—	—	—	71	81	—	—	84	61	41	76	62
15	*95*	81	73	66	78	67	55	94	67	59	70	62
20	—	—	—	71	82	74	52	82	70	59	72	70
25	*75*	57	82	61	86	74	50	66	79	57	72	70
30	—	—	—	82	88	66	54	89	69	54	79	80
40	—	—	—	—	72	61	—	—	—	—	—	—
50	*75*	63	82	75	70	63	55	74	68	55	81	73
60	*95*	81	—	—	—	—	—	—	—	—	—	—
70	*88*	62	—	—	66	64	55	82	73	55	82	79

* The colour was not grey or grey brown but light buff to chocolate, qualitatively similar to the October 1952 sample.

† The samples are comparable among themselves, but were wet and so more transparent and appeared too dark.

VISUAL RANGE OF THE SECCHI DISC IN RELATION TO THE PHYTOPLANKTON

It is known that the visual range of an object is affected by the illumination received by the intervening medium, air or water, as with strong illumination more light is scattered in the line of sight. We are indebted to Dr H. H. Poole for pointing out that determinations of visual range, when the line of sight is shaded from sunlight by the hull of a ship, are of necessity higher than when not thus shaded. However, the shading of the range is here not from direct sunlight, as in air, but from sunlight much reduced in intensity by passage through the water and with some surface loss. There is, on the other hand, a large reduction in visual range when the sunlit surface of the sea is interposed between the eye and the object. It seems preferable, therefore, to accept readings of the range made through a shaded water surface.

Thus on 12 April 1948, the sky was cloudless and the sea surface glassy; the disc was seen to 17 m in the sun, but to 21 m in the shade. Under the same conditions on 10 May the depths were, respectively 19 and 22 m. In more opaque water at the same station, E 1, the sun and shade readings were 6·5 and 7·7 m. Care was taken to have several youthful observers. It is obviously more difficult to see this small disc when the water is rough, but with patience consistent readings can be got. The colour seen here is greenish for 9 m and a lovely blue for 21 m.

The visual range of the Secchi disc has been determined for many years. The results using a 20 cm disc at Station E 1 from 1948 to 1953 are shown in Table III.

TABLE III. VISUAL RANGE OF 20 CM SECCHI DISC AT STATION E1

D, date; M, range in metres. Observations in shadow of ship.

	1948		1949		1950		1951		1952		1953	
	D	M	D	M	D	M	D	M	D	M	D	M
Jan.	—	—	—	—	18	11	24	*11*	7	*12*	21	*16*
Feb.	2	*8*	—	—	—	—	26	*13*	5	*14·5*	23	*15*
Mar.	3	*16*	1	*18*	—	—	—	—	11	*12*	16	*13*
Mar.	10	*17·5*	—	—	24	*13*	28	*10*	—	—	25	*16*
Apr.	12	*21*	13	*13*	—	—	—	—	16	*13*	13	*14*
Apr.	—	—	—	—	—	—	25	*14*	—	—	27	*11*
May	10	**22**	9	*14·5*	3	*13*	17	*20·5*	15	9	11	7·5
June	9	9	6	*19·5*	—	—	—	—	—	—	8	**22**
June	15	*10*	—	—	—	—	—	—	19	**23**	22	*11*
July	—	—	4	**21**	19	**15**	17	**22**	15	*18*	20	*11*
July	—	—	8	*16*	—	—	—	—	—	—	—	—
Aug.	16	7·5	17	*18*	23	*14*	22	*14*	20	*11*	10	*17*
Aug.	—	—	29	*17*	—	—	—	—	—	—	—	—
Sept.	—	—	—	—	20	*13*	27	9	22	*18*	8	*11*
Oct.	5	*12*	6	*11*	23	*14*	24	*18*	21	*15*	5	*12**
Oct.	—	—	—	—	—	—	—	—	—	—	20	*18*
Nov.	—	—	8	9	22	11	27	*13*	20	*14·5*	17	*17*
Dec.	—	—	—	—	—	—	—	—	22	*14*	14	*16*

Depths are shown in italic, save maxima, in bold, and minima, in roman type.

* 1 m added to correct for observation having been made in weak sunshine.

Several workers have presented results for phytoplankton and visual range of the Secchi disc which indicate an inverse relation between the two sets of results. Thus during the Great Barrier Reef expedition Marshall (1933) showed the 20 cm disc depths and number of diatoms per litre. The figures relate to hauls with the fine silk tow-net and wind action on the shallow water was a serious disturbing factor. Nevertheless, in her 1-year curve seven marked decreases in the disc readings coincided with high diatom numbers.

During the same expedition Russell & Colman (1934) found that the curve for total copepods taken in oblique hauls of the silk net follows closely that for the depth at which the disc disappeared. The peaks in the plankton curve occurred when the water was clearest. They considered that this was due to the copepods, etc., having moved down when the light passing through very clear water became too bright. This seems very probable, but the absence of the animals may in itself have contributed to the clearness, though the larger the animal is, the less is its obscuring effect over a large area, since finely divided matter has a greater surface, weight for weight.

Pettersson, too (1934*a*), cited phytoplankton figures to support a direct relation with the scattering of light, determined with his new scattering meter. The diatoms were centrifuged, identified, and counted by H. Högland, who in later work instead of centrifuging allowed the cells to settle. Atkins & Poole (1933) measured the extinction coefficient in spring just when phosphate consumption indicated that a great increase in the phytoplankton had already occurred. They found it low. Evidence has since been obtained which shows that such clearing of the water may be due to the sinking of the diatoms and to their consumption by animals. Further, the extinction coefficient is not largely affected by scattering when it is determined by measuring the illumination falling on horizontal plates, since light scattered out of the direct beam is compensated for by light scattered in.

There is no doubt that water masses may be characterized by the extinction coefficient. This has been shown by Jerlov & Koczy (1947) and in much detail by Jerlov (1950, 1951) also by Krey (1952). Joseph (1950) has recorded changes in extinction while the ship steamed ahead. When, however, we are dealing with a stable water mass, or one the movements of which are within an area experiencing closely similar meteorological conditions, it is then profitable to study the changes in visual range as a rapid and simple indication of changes in phytoplankton. Riley (1941) recorded this inverse relation, a rich plankton with Secchi disc 5·5–8·5 m, even down to 3·5 in April, and a lesser amount of plankton with 12·5 to 19 m. Riley followed the changes in the chlorophyll content of the matter filtered out, as has since been done by Gessner (1944) and others.

Fig. 2 shows the visual range in metres of the 20 cm Secchi disc at Station E 1 from September 1951 to November 1953, the scale being on the right-hand ordinate. On the left is the scale for chlorophyll in milligrams per m^2 in the surface to 20 m water column, 1 m^2 in area. Very strikingly the minimum visual range, 7·5 m, was observed on 11 May 1953, when the chlorophyll from 0 to 20 m was almost at a maximum. On 16 August 1948, 7·5 m was also recorded, but nothing is known of the chlorophyll or plankton for that day. But on 8 June 1953 the disc was seen to 22 m with the minimum amount of chlorophyll. The clearest water for the years 1948 onwards was observed on 19 June 1952, with 23 m and only 37 mg chlorophyll in the column. But by 22 September 1952 the chlorophyll had risen to 231 mg as a result of the growth consequent upon vertical mixing which brought up phosphate, etc. The visual range had also risen, after having been down to 11 m in August, up to 18 m in September; it would not have been so high but for the recent mixing with the deeper water poor in plankton. One cannot therefore say that an increase in the visual range necessarily indicates a decrease in phytoplankton.

There was always watchfulness to see whether such changes in visual range were due to clearer oceanic water having moved into or across the English Channel. But salinity determinations gave no evidence in favour of such movements at the dates concerned. Thus on 27 April and 11 May with high chlorophyll the 0–20 m salinity averaged 35·18 and 35·16, whereas with minimum chlorophyll on 8 June the salinity averaged 35·13 at Station E 1. The very slight decrease in salinity is accounted for by dilution with river water.

Again, between 5 and 20 October 1953, the whole isothermal water column at E 1 rose 0·50° C. This might be taken to show that warmer water had come

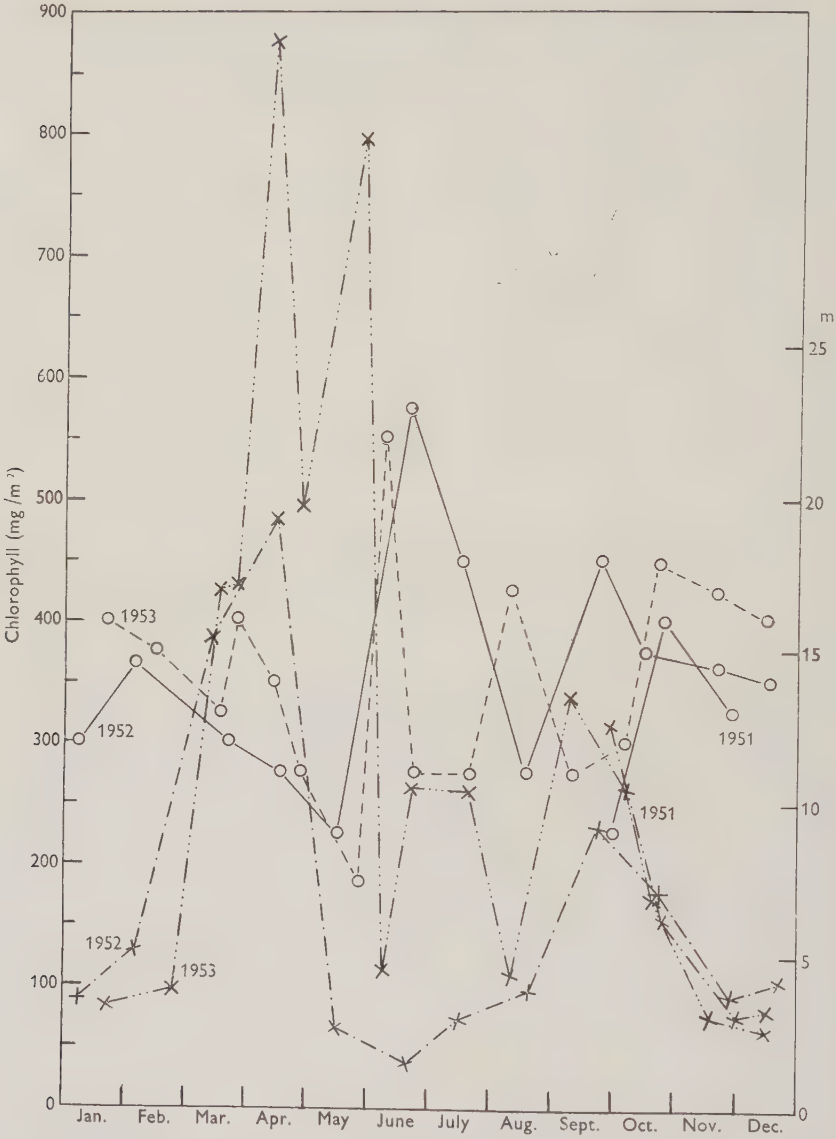

Fig. 2. The left-hand ordinates show chlorophyll in milligrams, the content of a column of sea water at Station E1, of area 1 m^2 and depth 20 m. The right-hand ordinates show the visual range of the 20 cm diameter Secchi disc. The abscissae show months. Chlorophyll is shown by a dash and two dots for 1951 September to November; for 1952 by a dash and dot line; for 1953 by a dash and three dots. Visual ranges for 1951 September to November and for 1952 are shown by full lines, for 1953 by a broken line.

up from the south. But the occurrence was probably due to a local warming during a very sunny period, 4·78 h sunshine daily average, as against 2·62 for the rest of October, the monthly averages for which have been (1950) 3·47 h, (1951) 4·38 h, (1952) 3·19 h, and (1953) 3·73 h. Furthermore, the salinity at E1 was, as average 0–20 m, 35·22‰ on 5 October and 35·21‰ on 20 October with identical surface values nearer shore at Station L4 (about 5 miles from Plymouth Sound). The effect of the Sound water had pushed out farther during the summer months, but its influence upon visual range appears to have been negligible as compared with that of phytoplankton increase or decrease.

A low visual range in winter, or in exceptionally stormy weather, may be merely an indication of the stirring up of bottom deposits. Judging by the filter-disc photographs and albedo determinations, turbidity arising from the bottom must be very rare at E1, in weather when it is possible for the ship to work, save possibly during the isothermal mixing period. The bottom gave a relatively dark disc on 25 March 1953, due to a rich phytoplankton, but did so on 20 July because the whole column was remarkably poor in suspended matter. Marshall & Orr long ago (1928) reported that 'during the spring months it was not uncommon in shallow water to find more plankton near the bottom than anywhere else'. They also found that during the spring outburst the phytoplankton caused such a great reduction in submarine illumination that the compensation point (or depth) was at times raised to 5 m or less.

We are indebted to the captains, crews and naturalists in charge of the Research Vessels *Sabella* and *Sula* for the samples, temperatures and visual range observations, also to Mr F. A. J. Armstrong for filtering some of the samples, and to Dr H. H. Poole for valuable criticisms.

SUMMARY

When sea water of Station E1 is filtered through membranes of average pore diameter about 1 μ the suspended matter, organic and inorganic, is usually seen to be greatest at the surface, occasionally at 25 m or other intermediate depths, and but rarely at the bottom, 70 m. High amounts, elsewhere than at the surface, are due to phytoplankton. The suspended matter, after ignition, was 1·6 parts per million at the surface in a June sample, with 1·0 at 5–10 m and 0·4 p.p.m. below. Values given previously, based on surface samples only, are too high.

Microscopic examination of the discs, carried out by Dr A. G. Lowndes, shows that the ground mass is fine clay. Fibres from woods, ropes and twines were also seen and black carbonaceous specks volatilized by heating the slide. There were also diatoms, copepods, sponge spicules, a small amount of kaolinite, specks of iron oxide, quartz grains, cordierite, micaceous matter probably sericite, tourmaline and broken grains of felspar (orthoclase).

Fragments of shell were common. Most of the grains were under 40μ in diameter.

Comparison between successive months can be made from photographs of the discs, but more accurately by determining the relative albedo with a photoelectric cell. The lowest albedo was 25%, for 21 January. The lowest column values of the albedo were for 5 October, when the water had just become isothermal, and the 5 m interval mean, with appropriate interpolation, gave relative albedo 54, with similarly 64 for January and 81 for August. But the effects for mineral matter and plankton are not truly additive.

There is an inverse relation between the amount of phytoplankton determined by spectrophotometric analysis of chlorophyll extracts, and the visual range of the 20 cm Secchi disc, though admixture of suspended inorganic matter may at times disturb the relation. Very clear water is always poor in phytoplankton. Water unusually turbid for the locality is probably rich. The conclusions are based on a study of Station E 1 for two years and a quarter.

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J. Mar. biol. Ass. U.K. (1954) 33, 511–514
Printed in Great Britain

A NOTE ON THE ISOLATION OF SMALL MARINE ALGAE AND FLAGELLATES FOR PURE CULTURES

By M. R. Droop
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Increasing interest in the comparative biochemistry of marine algae and protozoa is creating a demand for experimental material in the form of pure, i.e. bacteria-free, cultures. For nutritional and biochemical studies pure cultures are, of course, obligatory; but the number of marine strains available for this sort of work is lamentably small. Indeed, none of the so-called μ-flagellates has hitherto been grown in pure culture as far as I know, although there exist pure strains of some of the larger and hardier forms.

Hardier species of motile or non-motile algae can be plated out by normal bacteriological techniques, provided that the material is growing sufficiently vigorously for there to be cells without attached bacteria. Marine examples of this class are species of *Chlorella*, *Chlamydomonas*, *Brachiomonas*, *Platymonas*, *Nannochloris*, *Phaeodactylum*, etc., many of which can be maintained on agar. For plating, I have found that test-tube slants (1 % agar) are preferable to Petri dishes, since drying out of the medium is then delayed; but slants require more skill for the actual isolation than do plates.

The use of antibiotics seems, except with filamentous or gelatinous algae, to be applicable only to species which can be handled by simpler methods. This has recently been demonstrated by Spencer (1952).

Allen & Nelson's (1910) time-honoured 'dilution' method has been used with great success by Gross (1937), Parke (1949) and Butcher (1952) for obtaining unialgal cultures of many interesting marine species; but it is doubtful if pure cultures could ever be obtained by serial dilutions, since bacteria usually occur in far greater numbers than algae.

A method of purifying flagellates which is widely used among freshwater workers is the 'pipetting' or 'washing' method as developed by Pringsheim (1946). I want to show that, with very little modification, Pringsheim's technique can be used with success for some μ-flagellates.

The washing method has two advantages over all other methods; one is of principle, that the organism to be isolated is selected by the worker and not by the medium; the other is a practical one, that the ratio of bacteria to algae is reduced by astronomical proportions at each manipulation, which is not the case with serial dilutions. Like the plating method it depends for its success on the cells being free from attached bacteria in the first place. Motile forms are mostly clean and therefore present no problem. With non-motile or mucilagenous species it is necessary to employ vigorously growing populations. Then, even such gelatinous algae as the freshwater *Paulschulzia pseudovolvox* may be purified by washing, and indeed, I have also washed the diatom *Phaeodactylum tricornutum* successfully.

Pringsheim's technique has been described in some detail in his book (1946) and in various articles (1950, 1951). The apparatus consists of a binocular dissecting microscope with a cardboard breath-guard; a store of sterilized Petri dishes, each with a watch-glass inside; micro-pipettes; and an appropriate variety of sterile culture media in test-tubes. Micro-pipettes are made simply by: (i) plugging a 9 in. length of $\frac{3}{8}$ in. glass tubing at both ends with cotton-wool; (ii) drawing this out in the centre to make two long-nosed pipettes with a 1 mm bore at the narrow end, these to be sterilized and stored sterile; (iii) immediately before use, further drawing out the thin part of the pipette to a bore of about 0·1 mm in a very narrow flame, and breaking the capillary tube cleanly at a convenient length by a sharp longitudinal tug with flamed forceps, an operation to be repeated before every manipulation to ensure sterility; and (iv) attaching a piece of rubber tubing, glass-stoppered at the free end, which serves to eject liquid from the pipette, and also has the necessary strength to overcome the strong capillary force in the tube.

Into the first of a series of six of the watch-glass combinations, each containing about 3 ml. of sterile culture medium, is placed a drop of material containing the desired organism. One normally starts with the highest powers of the binocular for identification, but once the look of the organism and its characteristic movement have been learned it is possible to go to the lower powers for isolation. Single cells are selected and drawn into the pipette by capillarity and transferred to the second glass of the series. For small organisms it is convenient to watch them being ejected into the new medium, as this may save a little time in searching. In any case they should be allowed to swim around for a while before they are picked up again, so that they become washed of the old medium. This treatment is repeated, until after the final washing they are transferred, one to each culture tube.

This method, as it stands is applicable down to about 15μ; but for smaller cells it becomes too tedious. I have used it in purifying cultures of *Oxyrrhis*, *Chlamydomonas*, *Brachiomonas*, *Platymonas*, *Syracosphaera*, etc.

It is possible to make use of phototactic responses for the purification of some μ-flagellates. This avoids most of the difficulties arising from their small size. For example, an organism of 5μ can be washed with less trouble with the aid of phototaxis than one of 50μ handled in the normal way; but of course success depends on one's being able to induce phototaxis. Luckily, many species seem to exhibit a very strong negative taxis when they are transferred from the wild to a culture medium. All that is needed is to put a drop of the material gently at the window side of the watch-glass and wait 5 min (or perhaps 10) when the flagellates will have congregated at the side away from the light, leaving most of the unwanted bacteria on the other side. From here the flagellates are transferred *en masse* with a rather large-bore micro-pipette to the window side of the next watch-glass, and so on until the last. Then under medium power of the binocular the flagellates, mere specks, are picked up singly with a fine-bore micro-pipette and placed in culture tubes. The last operation is very rapid: fifty isolations can be made in half an hour or so. The proportion of contaminated to clean cultures obtained with six washes is about 1:10.

I do not know what the size limit of this method is; but provided an organism is visible, if only as a speck, it is possible to pick it up. The essentials are that there is a preponderance of the desired flagellate in one's material to begin with, and secondly that it is motile and phototactic.

My successes during last summer with this method include two members of the Chrysophyceae, *Monochrysis lutheri* and *Prymnesium parvum*; one of the Cryptophyceae, *Hemiselmis* sp., and several Chlorophyceae, all below 10μ. As an example of the efficiency of the procedure, *Monochrysis lutheri* (5–7μ) was found at a cell concentration of 16,000,000 per ml. in a pool of salinity 25‰. One day was devoted to preparing the media and isolating the organism. Forty-eight isolations were put into twelve different combinations of medium; and out of this forty-eight, ten flagellate cultures and three bacterial cultures resulted. All the successful ones contained glucose and liver extract. *Hemiselmis* (6μ), on the other hand, was more difficult. The natural material was at a cell concentration of about 30,000,000 per ml. from a pool of low salinity. I made three attempts on the same scale as for *Monochrysis*. In the first I ended up with a large number of pure cultures of a minute heterothallic *Chlamydomonas*; not until the third attempt did I obtain any growth of *Hemiselmis*. Then only three cultures resulted of which two were bacteria-free.

In general there is no difficulty in ridding a motile organism of bacteria; it is getting it to grow by itself which often presents problems. Synthetic media are inappropriate for preliminary studies, even for the few species which are known to grow in such media. It is often necessary and always safer to use natural extracts, natural sea water, soil extract, etc. Moreover, since the aim is bacteria-free cultures there is nothing to prevent the use of yeast, beef and liver extracts as well (Pringsheim, 1946).

The flagellate is probably phototrophic, but there is a strong possibility that it has one or more heterotrophic requirements in addition; these are best met by including extracts of organic materials, liver and yeast being the most efficient.

I find it convenient to prepare all stock media with an artificial sea water, sterilize them, then mix them with equal proportions of separately autoclaved natural sea water. In this way many combinations of yeast, beef, soil extracts, peptones, etc., and other nutrients having various pH's can be quickly prepared, while at the same time ensuring a high proportion of natural sea water. Unautoclaved, but Seitz-filtered, media can also be included. The idea is to use a large range of media and isolate a large number of cells on the chance that one of the combinations will be suitable.

One word about sterility testing. As a routine, four tests are made: (i) fresh-water liquid, (ii) fresh-water agar, (iii) salt-water liquid, and (iv) salt-water agar. The medium contains yeast, beef, and soil extracts, glucose and acetate. In addition, all cultures are maintained, as far as possible, in organic media so that chance contamination becomes visible.

All the species which have been mentioned here are supra-littoral or neritic forms, and it is probable that they are easier to handle than the pelagic ones. Nevertheless, the μ-flagellates among them are delicate: they need natural sea water for their growth and show the usual tendency to burst at the least provocation. It is possible that pelagic species could be handled in the same way as the littoral forms. I have no doubt that, wherever feasible, physical manipulation is the surest and quickest means of obtaining pure cultures, and in my view it is also the easiest. The object of this note has been to record that its range of application has been extended to include some organisms as small as 5μ.

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J. Mar. biol. Ass. U.K. (1954) 33, 515–536
Printed in Great Britain

THE ABUNDANCE OF *OCTOPUS* IN THE ENGLISH CHANNEL

By W. J. Rees and J. R. Lumby

(Text-figs. 1–5)

During 1950, the Common Octopus (*Octopus vulgaris* Lamarck) was to be found along the south coast of England in greater numbers than at any time since Garstang (1900) reported on the 'plague' on the coasts of Devon and Cornwall in 1899–1900.

In earlier papers (Rees, 1950, 1952) the distribution of the octopus in our northern waters was reviewed, and it was demonstrated that this species is an immigrant which breeds on our south coast only rarely. It reaches these coasts by being brought there as a planktonic larva by the water circulation in the English Channel and by migrations of the adult. The most important factor in controlling the movements of the adult, however, might be expected to be the water temperature in the English Channel—where the species is at the northern limit of its breeding range and might therefore be extremely sensitive to slight changes in temperature.

The earlier paper (1950) was completed in January of that year, at a time when fishermen in the Channel Islands were complaining of the abundance of octopus in their area. It then seemed likely (a surmise which proved correct) that the octopus might also be more abundant on the English coast in the summer, if events followed the same course as in 1899–1900. This paper deals primarily with its abundance in 1950 and subsequently. It is now possible to present a much better picture of the distribution of larvae based on the pilchard-egg investigations of the Ministry of Agriculture and Fisheries. More information is also available on water temperature as a controlling factor, and the possible seasonal migration of the adult in normal and abnormal years is considered.

The Abundance of the Adult

Early in 1950 there was no evidence that *Octopus* would be abundant on the English coast, but the presence of the species in numbers in the Channel Islands in January was an indication that a repetition of the events of 1899–1900 was a distinct possibility. In March 1950, however, an occasional juvenile *Octopus* was caught by the research vessels of the Marine Biological Association off Plymouth. One of these, a specimen with an arm span of 70 mm and a ventral mantle length of 14 mm, was kindly sent to us by

Mr F. S. Russell. These can be assumed to have been spawned in the previous July or August and to be about 7–8 months old.

Apart from juveniles, the first indication of real abundance came from the Devon area. The trawler *Girl Vine* (Skipper T. Harvey), working out of Torquay in the third week of May in a position 20 miles S.S.E. of Berry Head, reported that in one haul the cod-end of the trawl was full of *Octopus*. These were large with a span of about 3 ft. A few octopus were also taken in other hauls made during the same fishing trip. Fourteen days later on the same grounds octopus were not noticeably abundant; later events proved that they had moved inshore.

During the last week in May an octopus with an arm span of about 30 in. was taken in a crab pot at Bexhill by a fisherman (Mr J. Easton). In the first half of June octopus began taking lobsters and crabs from baited pots in Start Bay and Babbacombe. As the season advanced Babbacombe fishermen were taking 30–40 a day in their pots. At Beer and Seaton, the shell-fishery was almost abandoned for the same reason. The area between Seaton and Brixham appears to have been affected the most, but farther west some Plymouth fishermen abandoned shell-fishing because the octopus were taking their catches. For the first time in many years the Plymouth Laboratory was able to obtain octopus in sufficient numbers for experimental work.

During August a large number of octopus entered Dartmouth Harbour. Most of these were large with an arm-span of 4 ft.; some were caught by hook and line. Fishermen reported seeing young octopus of very small size during the autumn in the south Devon area.

On the north coast of Devon *Octopus* were not noticeably abundant, but a few were caught off Lundy Island. Towards the middle of October few octopus were being caught on the south Devon coast.

On other parts of the south coast of England *Octopus* was also common. In mid-August specimens 2½–3 ft. in arm-span were being caught in some numbers by anglers at Eastbourne and Brighton, and during the first week of September octopus of all sizes up to 4 ft. 6 in. in arm-span were being caught in lobster pots on Hoo Bank off Selsey Bill. There was an interesting record of an octopus (30 in. in arm-span) from Deal on 2 September; this specimen, now in the British Museum (Natural History), is one of the few ever recorded from the North Sea.

Octopus were being caught throughout the winter (1950–51) by the R.V. *Sabella* operating within 16 miles of Plymouth Breakwater (see Appendix 1 by Lieut.-Commander C. A. Hoodless, D.S.C., R.N.R.). It is interesting to note that this ship caught its first octopus on 4 September 1950, the totals for September, October, and November being 2, 3 and 2. These small numbers probably give an accurate impression of the situation on offshore grounds and serve to emphasize that the main body of octopus was close inshore. One Plymouth fisherman, who had seen only four octopus in 35 years of fishing, caught no fewer than fifty in and on his lobster pots by the first week in October. The increase in catches in winter and spring suggests that the octopus, like *Eledone*, moves into deeper water after the summer.

In Jersey, too, the octopus was numerous in 1950, and there appears to have been no reduction in numbers in 1951 and in 1952. On the French coast also, during the summer of 1950, *Octopus* appears to have been very abundant both in the neighbourhood of Cherbourg and on the north coast of Brittany.

Prof. J. Z. Young, F.R.S., has kindly allowed us to publish some notes on the situation in the Cherbourg area in September 1950. He states: 'There were considerable signs of a plague, at least on the stretch from Cherbourg to the tip of the Hague peninsula. On 4 September I saw a great many of them lying about in the port at Cherbourg; they were being caught by boys and were apparently unsaleable, the fishermen were baiting their lines with pieces of tentacle. On 11 September I was still more struck with their abundance at Omonville. There is a little harbour there and the jetty runs out among the rocks. Looking down among these we could see a great number of *Octopus* moving about in the water at high tide. For instance, at one moment we counted no less than twenty-five in sight, even though the sea was rough and vision was limited to a few yards. Of course I cannot say whether they would be equally abundant away from the jetty, perhaps they had come in for food. They were certainly very hungry and attacked pieces of dead octopus as soon as lowered into the water; in fact the boys were fishing for them in this way and once the dead octopus had been seized the live ones would hang on to it and could be drawn out of the water on to the jetty. In this way one could pull them out one after the other almost as fast as the bait could be lowered into the water.

'Elsewhere along the coast there were signs of abundance though I did not actually see such large numbers anywhere else. However at low water they were caught by prodding under rocks at Urville on several days.'

Some additional information published in *La Pêche Maritime*, Nos. 868, 869 and 870 (1950) is quoted below:

May 1950: Portsall. 'La pêche aux crustacés (homards et langoustes) a été d'un rendement à peu près nul et d'ailleurs la côte à un tel point infestée de pieuvres qu'il devient difficile de preserver le crustacé dans les casiers et viviers où ces mollusques parviennent toujours à s'infiltrer.'

July 1950. 'Les pieuvres très nombreuses, certaines d'assez grandes dimensions, pénétrant jusque dans les concessions ostréicoles, font des sensibles ravages. Les baies ont été visitées par de nombreux crabes-araignées d'un calibre respectable, mais se déplaçant rapidement, au grand dam des pêcheurs.'

August 1950: Paimpol. 'Les pieuvres sont plus nombreuses que jamais; on les trouve même dans les concessions ostréicoles. Les crabes-araignées ont été parfois abondants dans les baies, se déplaçant jusque dans les chenaux.'

It is thus evident that the octopus was even more abundant in 1950 on the French coast than on the English side.

PLANKTONIC LARVAE IN 1948, 1949 AND 1950

In an earlier report the Channel larvae from cruises in 1948 and 1949 were plotted on a single chart (Rees, 1950, fig. 2). The results for these two years have been re-plotted on separate charts, and results from the pilchard-egg cruises of the Ministry of Agriculture and Fisheries research vessel *Sir Lancelot* have been added for 1949. The results for 1950 are based almost entirely on the catches of the *Sir Lancelot*.

The year 1948. In this year only the collections made by the M.Y. *Manihine* in the eastern Channel and over the eastern end of the Hurd Deep were available (Fig. 1, 1948). Young larvae found at five stations at the end of August are noted in Table I.

TABLE I. CATCHES AND LENGTHS OF YOUNG *OCTOPUS* LARVAE

Station	Catch	Ventral mantle length in mm
47	4	2·55, 2·85, 3·15, 3·15
48	7	1·5, 1·65, 1·8, 2·25, 2·4, 3·0, 3·0
49	5	2·25, 2·4, 2·85, 3·0, 3·0
50	4	1·65, 2·4, 2·7, 2·85
51	4	2·10, 2·55, 2·85, 3·0

The positions of these stations have already been given (Rees, 1950, p. 368). There was no information on distribution to the west and no larvae were taken at St. 45 (the southernmost of a line running across the Channel from south of the Isle of Wight towards the Cherbourg peninsula). Two larvae at St. 48 and another at St. 50 were at or just about hatching size; they could not have been many days old,[1] and it could be inferred that the breeding grounds were not far away.

The year 1949. During this year plankton hauls were again taken in the central area of the Channel by the *Manihine* in August and *Octopus* larvae obtained both to the east and the west of the previous positions (Rees, 1950, p. 368). However, a much more complete picture of distribution during June and September was made possible by an examination of the plankton hauls taken by the *Sir Lancelot*. These cruises covered the whole of the Channel and, although intended for another purpose, yielded valuable information on where larvae could be found during these particular months (Fig. 1, 1949). Larvae were found at three *Sir Lancelot* stations (39, 45 and 46) in June off the north coast of Brittany. No records are available for July and only those of the *Manihine* for August. The apparent absence of larvae in the central Channel to the north of Alderney is thus due to absence of records. The September cruise of the *Sir Lancelot* yielded larvae at five stations; all were farther away from the breeding grounds, on the north coast of France and the Channel Islands, than recorded earlier in the year, and must be presumed to have been dispersed by water movements. Larvae at two stations near the English coast appear to be sufficiently far north to settle down on offshore grounds and to work their way inshore during the following year.

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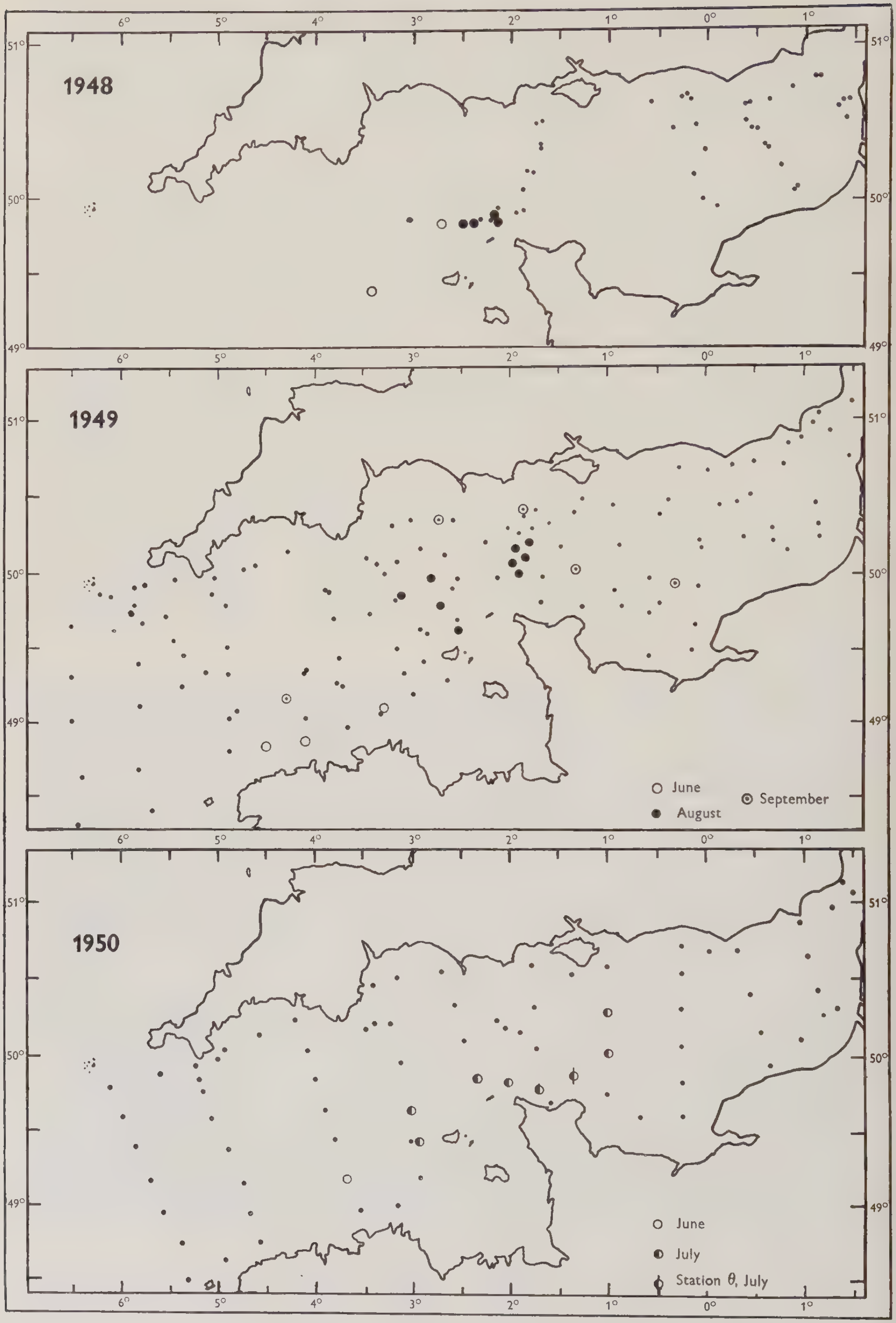
[1] It is assumed here that larvae would hatch at about 1·5 mm (in ventral mantle length) as at Naples.

The year 1950. The pilchard-egg cruises of the *Sir Lancelot* were continued during April, May, June, July and August. No *Octopus* larvae were found in April and May, but in June two larvae were obtained at a station to the north of Roscoff. The July cruise yielded the only distribution picture we have for this month, and it is significant that all the larvae (except those at the two easternmost stations) were found fairly close to the Channel Islands and the Cherbourg Peninsula (Fig. 1, 1950). It is reasonable to assume that the larvae at Sts. 33 and 34 in mid-Channel were being transported fairly rapidly in an easterly direction. The series of night hauls at St. θ revealed the presence of larvae in no fewer than fourteen hauls (the maximum catch being seven and the minimum one), from which we can infer that larvae were by no means rare and that hatching from the eggs was in full swing. From the size of the larvae it can be assumed that they had been hatched at the end of June and the beginning of July; but most of them were small and probably not more than 7–14 days old.

In August (as shown separately in Fig. 1) larvae were found at all but three of the stations worked on the south side of the Channel from Ushant to approximately 1° W. There was also an isolated larva at St. 15 off the Cornish coast which presents an intriguing problem, whether it was carried across the Channel from the region of Ushant by the current or whether it was spawned locally (but see p. 522). This picture for August indicates that larvae were widely spread during this year, but unfortunately there are no records suitable for comparison, so that we do not know whether this is the normal pattern of distribution for this month or whether it is only an indication of widespread successful hatching of larvae associated with the plague years of 1949–50. These are the last detailed records available to us.

The records indicate that hatching begins in June on the north coast of Brittany and by July larvae are more common in the mid-Channel area and around the Channel Islands. In August (if we are to judge by the years for which we have records) larvae are more abundant and more widespread than during earlier months, while in September fewer larvae are planktonic and they are farther north and east in distribution than in any other month.

A comparison of relative abundance of larvae during these years, especially as 1950 was a 'plague' year, would have been of great interest. However, there were no *Sir Lancelot* cruises for July 1949 (to compare with July 1950),


1948
1949
1950
6°
5°
4°
3°
2°
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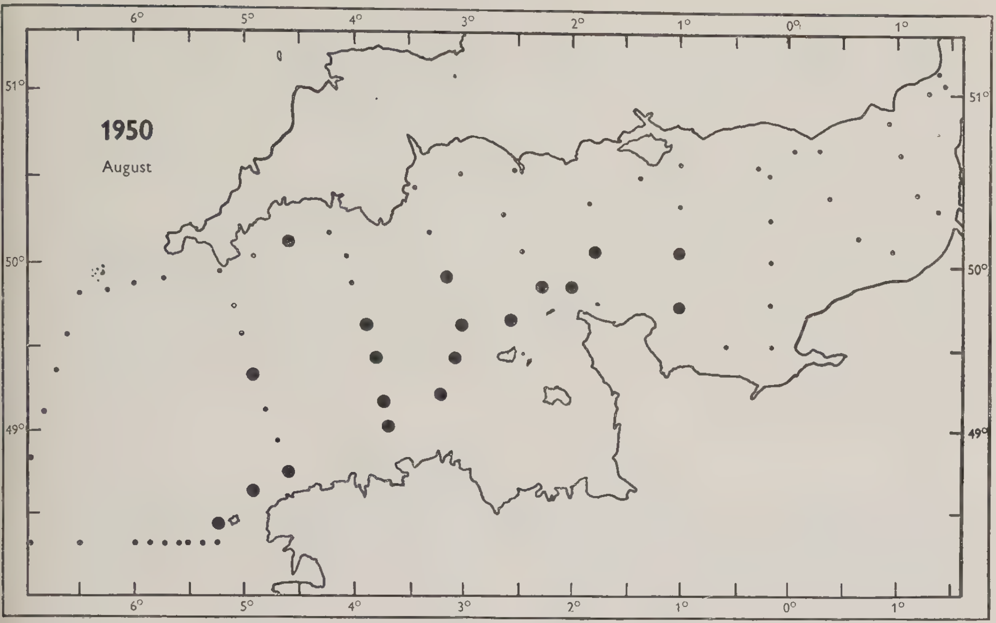
0°
1°
51°
50°
49°
○ June
● August
⊙ September
○ June
◐ July
Station θ, July



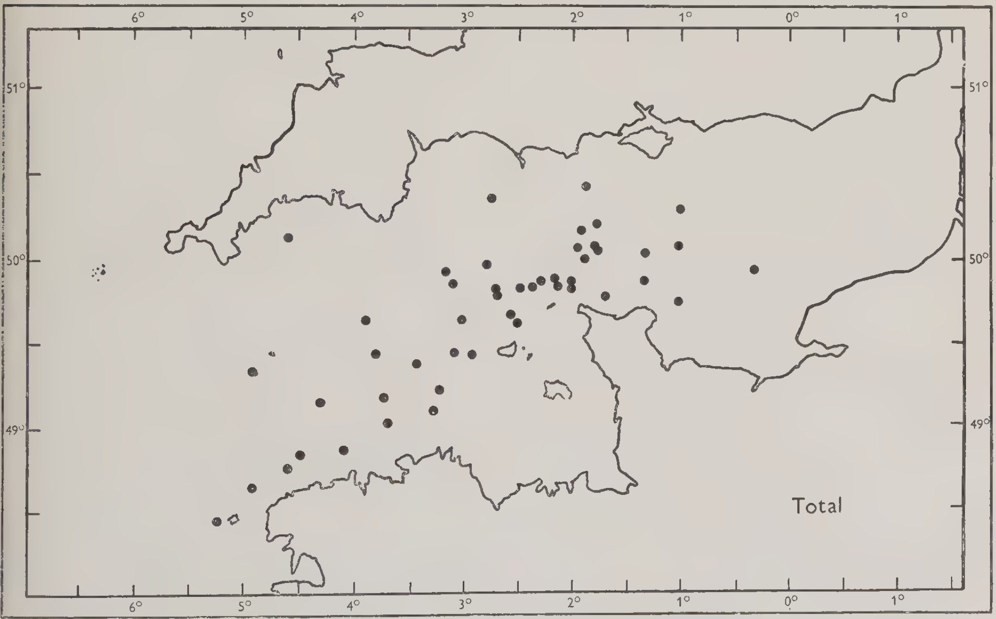


Fig. 1. The distribution of *Octopus* larvae 1948–50. Stations worked during the period in question are marked with a dot. Circles denote stations at which larvae were taken, as specified on the separate maps. The two open circles on the 1948 map denote positions at which the Danish research vessel *Thor* took larvae in 1906 and 1910. These positions are also included in the last map which gives an aggregate of positions at which larvae were taken.

August 1949 (to compare with August 1950), and September 1950 (to compare with September 1949); thus a comparison of the distribution and abundance of larvae for the two years cannot be made. As already mentioned, the distribution for September (1949 only) indicates that these late larvae are being transported to the north and east of the area in which they were hatched.

The total records of larvae for the three years (1948–50) and two records from the *Thor* expedition (Rees, 1953, p. 216) are also plotted in Fig. 1). These confirm earlier deductions that the main breeding grounds of *Octopus* are in the south-western area of the Channel and around the Channel Islands (Rees, 1950, p. 374). There is no indication that there is any extensive breeding in our inshore waters, even in years of abundance, and the only record of a larva in home waters in August 1950 from St. 15 of the *Sir Lancelot*'s cruise in that month cannot be positively certified as having been hatched in that area.

INFLUENCE OF SEA TEMPERATURE

Since Garstang (1900) drew attention to the possible role played by water temperature, many more observations of temperature have accumulated, which may be considered in relation to the new information on the various stages in the life history of *Octopus*.

Temperature at Time of Spawning and Hatching

Information on these initial stages is available from the cruises of the *Manihine* and *Sir Lancelot* in the English Channel in 1948, 1949 and 1950 (see pp. 518–22).

From a consideration of the ventral mantle lengths it is possible to estimate the age of the larvae taken. This length ranges from 1·5 to 6 mm, and the smallest larvae are evidently quite newly hatched, and not more than 2 or 3 days old. The age of the largest is put at about 2 months. From these data, the dates on which hatching took place may be estimated with considerable certainty for the youngest larvae, but with rather less for the larger specimens.

The time required for the eggs of *Octopus* to develop in the English Channel has not yet been established. In the present discussion a month is assumed to be the period of development in these waters. This assumption is further considered below (p. 526) and the period is more likely to have been under- than overestimated on this basis. As a rough approximation the date of spawning is taken as a month before the estimated date of hatching.

Octopus is believed to spawn only quite close inshore, within the 10 m depth line. (It is worth noting that the smallest larvae, 2 or 3 days old, were found at stations not more than 20–25 miles distant from the nearest coasts.) Only shallow-water temperatures therefore need be considered. Unfortunately the temperature data available for such inshore waters, particularly on the French side, are decidedly meagre. We are indebted to Mr H. J. Baal, President of the Société Jersiaise, for a series of monthly average temperatures at Jersey.

These are based on observations made at 9 a.m. daily at 1 m depth in the shade at the end of the Albert Pier, St Helier. For the western part of the Brittany coast, the only data available consist of the surface observations made near Ushant by commercial vessels crossing the mouth of the Channel, between Ushant and Land's End. The position to which the data used relate is approximately 48° 27′ N., 5° 15′ W., admittedly some distance off the mainland. In addition, there are the routine observations made by the commercial vessels on the Southampton-Channel Islands service, and by the research vessels of the Marine Biological Association off Plymouth. Except those for Jersey, these data are published in the Bulletins Hydrographiques issued by the International Council for the Exploration of the Sea, Copenhagen. They are illustrated, together with those for Jersey, for the relevant period, in Fig. 2.

The use of surface temperatures is justified in present circumstances, since at least in April and the early part of May, the waters of the English Channel may be expected to be homothermal (see Dietrich, 1950). Surface observations are thus reasonably reliable guides to the conditions on the sea bottom at this season, especially in the shallow waters inshore. Certain disadvantages may arise, however, from the fact that most of the data employed for this inquiry are in the form of monthly averages. In shallow water, the fluctuations of temperature are likely to be much greater than in the deeper water well clear of the land. It can be seen, for example, in Fig. 2 that the annual range tends to be considerably greater at Jersey than off Ushant, or even off Plymouth. Short-period fluctuations, on the other hand, are not to be discerned in the curves based on monthly averages. One or two very cold days in an otherwise mild month may have a disproportionately adverse effect on biological processes, while a few bursts of sunshine in an otherwise gloomy month may give a lot of encouragement to them. Such effects are likely to be masked by the use of monthly averages. However, other uncertainties involved in, for example, the dates of spawning and hatching and the places at which they occurred, are so numerous that it is doubtful whether we could profit by a closer examination of the temperature conditions.

During the greater part, if not all, of the season in which spawning, development, and hatching are taking place, the water temperature is in general rising. By estimating the dates, in the way described, on which the largest, and therefore the oldest, larvae taken were hatched and spawned, and reading the temperatures for these dates from the appropriate curves, we arrive at an estimate of the lowest water temperatures at which these events occurred. The values so obtained are shown in Table II.

The table indicates that in the years 1949 and 1950 spawning began in the first half of May on the western part of the Brittany coast, and in the latter half of the month in the Channel Islands district. For 1950, these dates are further supported by the fact that larvae were absent from the catches in

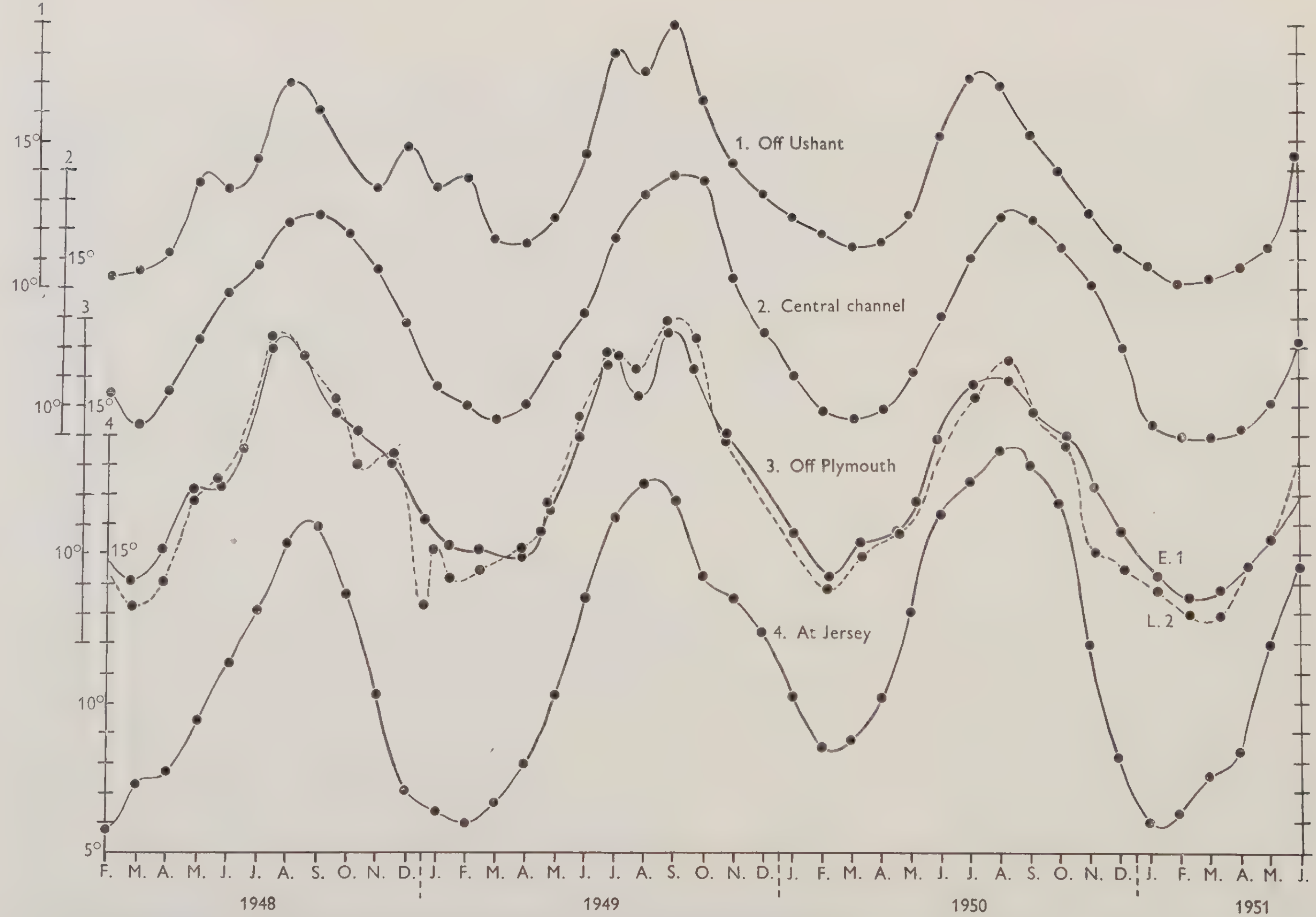


Fig. 2. The sea surface temperature (° C) at four localities in the English Channel from February 1948 to June 1951. The curves shown for the two positions off Plymouth are based on single observations; the other three curves are based on monthly mean values. The locality off Ushant (1) is Region 10 (see Lumby, 1935); the Central Channel (2) is a combination of Regions 20, 24 and 25; the observations off

April and May that year, and first appeared in June. The water temperature at first spawning is found to be about 11½° or 12° C.

Table II. The Estimated Dates and Presumed Localities of Spawning and Hatching, with the Appropriate Surface Water Temperatures (° C)

		Spawning			Hatching		
Cruise (1)	Locality (2)	Date (3)	*T*° C (4)		Date (5)	*T*° C (6)	
M., Aug. 1948	C.I.	7 July	12·6 (J.)	**12½**	7 Aug.	14·8 (J.)	**15**
S.L., June 1949	B.	18 May	10·5 (J.) 12·5 (U.) 11·8 (C.)	**11½**	18 June	13·7 (J.) 14·8 (U.) 13·4 (C.)	**14**
M., Aug. 1949	C.I.	1 June	12·0 (J.)	**12**	1 July	15·0 (J.)	**15**
S.L., Sept. 1949	B. & C.I.	7 July	15·5 (J.) 17·3 (U.) (C.)	**16**	7 Aug.	17·2 (J.) 17·3 (U.)	**17**
S.L., June 1950	B.	5 May	12·2 (J.) 12·1 (U.)	**12**	5 June	15·8 (J.) 14·2 (U.)	**15**
S.L., July 1950	C.I.	22 May	13·6 (J.)	**13½**	22 June	16·7 (J.)	**16½**
S.L., Aug. 1950	B. & C.I.	12 June	16·6 (J.) 15·1 (U.)	**16**	12 July	17·5 (J.) 17·1 (U.)	**17½**

In column 1, *M.* indicates *Manihine*; *S.L.*, *Sir Lancelot*. In column 2, C.I. indicates the Channel Islands region; B., the western part of the north coast of Brittany. In columns 4 and 6, J. indicates at Jersey; U., off Ushant; and C., the central Channel between the Isle of Wight and the Cotentin peninsula. The temperatures in heavy type are the round values considered acceptable. Spawning and hatching refer to the oldest larvae taken on each cruise.

Effect of Temperature on Date of Spawning

As regards the effect of temperature on the time when spawning begins, the possibility must not be overlooked that *Octopus* may start to spawn in the English Channel some time in May, mainly in response to an innate rhythm, with only very little regard to water temperature. The average temperature both at Jersey (for the years 1935–39, 1946–52) and also off Ushant (for the years 1928–51) reaches 11½° C at about the end of the first week in May. In separate years this value may be reached at Jersey as early as mid-April (e.g. 1933) or may not occur until mid-June (1948). If spawning is closely controlled by temperature, fluctuations of a similar order of magnitude must be expected in the dates on which the process starts. It is true that the results of the August cruise in 1948, a year with particularly low spring temperatures at Jersey, point in this direction, as they give a date for first spawning as late as July. But as the table shows, in 1949 and 1950, when there were sequences of cruises, the later the cruise, the later is the estimated date of first spawning. The explanation of this is not apparent, unless it be that by the time of the later cruises, the largest larvae may have already been so close to the bottom that they were not taken by the *Sir Lancelot*'s nets.[1]

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[1] The *Manihine* made horizontal hauls as close to the bottom as practicable, whereas the *Sir Lancelot*'s were vertical hauls.

There is, however, another feature which suggests that spawning began later in 1948 than in 1949 or 1950, namely, that the distribution of the larvae in August 1948 was not quite so wide as in the same month in the other two years. But not much reliance can be placed on this, since the western half of the Channel was not sampled in 1948. For the same reason it is doubtful whether we may conclude that the eggs spawned and larvae hatched in 1948 were fewer than in 1949 or 1950, as might at first sight appear to be the case. Moreover, these numbers may depend less upon temperature than upon the numbers of adults present. Thus the evidence at present available does not permit a decision to be reached on the question whether spawning may be expected to be earlier or later, or more or less intense, according as the water temperature is higher or lower.

Off Plymouth, the temperature of $11\frac{1}{2}^\circ$ C is reached on the average (1928–51) only some 2 weeks later than on the French coasts. If *Octopus* can tolerate as wide a variation in the date of commencement of spawning as 2 months, it is surprising that it spawns so very rarely on the English coast. This suggests that in regard to choice of spawning ground some factor other than temperature determines the animal's preference for the southern side of the Channel over the northern.

Accumulated Temperature and Development of the Egg

In the foregoing discussion, the assumption is made that hatching occurs 1 month after spawning. No direct confirmation of this, however, is obtainable from the evidence at hand. In so far as 'accumulated temperature' (Tait, 1951) is of importance to development, the requirements of *Octopus* estimated on this basis appear to be somewhat less in the English Channel than in the Mediterranean. For the English Channel, taking the temperature at spawning as $11\frac{1}{2}^\circ$ C and at hatching as $14\frac{1}{2}^\circ$ C, with 30 days for development, we obtain a figure of 390 day-degrees. For the Mediterranean, Portmann's figures (Portmann, 1933) give a value of 450 day-degrees, and if it is assumed that the temperature at Naples was between 15 and 20° C at the time of his experiments, Naef's figures (Naef, 1921, 1923, 1928) give values between 420 and 560 day-degrees. It is not considered that these figures conflict unduly with the assumption of at least a month for development in the English Channel.

Temperature and Abundance of Octopus

For the later stages of the life history of *Octopus*, we may consider the reports on its abundance in the English Channel at different periods. After about 1885 octopus were scarce on both French and English coasts, until in 1899 their numbers assumed plague proportions on the French side, and began to rise appreciably on the English side. In the following year, 1900, the octopus were so numerous as to form a nuisance on both sides of the Channel.

They were noted as plentiful on the Sussex coast in 1913, 1922 and 1948, and they were a plague on the Finistère coasts in 1922. These latter, except possibly that in 1922, may be regarded as more or less minor occurrences, not comparable in magnitude with those of 1899 and 1900. In 1950 a plague sprang up anew on the French coasts, and the number of octopus began to rise on the English side also during the same year. There were very large numbers of octopus present on both sides of the Channel in 1951, and on the French side in the following year also. Whether they had in fact disappeared from the English coasts in 1952 is not certain. Official records of the size of the octopus population are not kept and the information available cannot be claimed as complete and precise. Accordingly, the absence of reports of octopus in the newspapers, for example, may be due to the animal having lost its news value in 1952.

In his report on the plague that occurred at the turn of the century, Garstang (1900) suggested that the warm summers and mild winters which, he states, were experienced during the few years preceding the plague provided conditions suitable to a warm-water animal. He remarked further that 'the plague of octopus may be traced to the influence on the reproduction of this species of the exceptionally favourable conditions which prevailed in 1893'. With these points in mind, we may examine the considerable sequence of temperature data now available for the English Channel, in relation to the further records of octopus abundance. Surface observations are representative of the whole column at all seasons in the English Channel, except in the westernmost part. Here a thermocline develops at times during summer, with the result that the upper 30 m or so of water may be up to 5° C warmer than the water below (see Dietrich, 1950). The height of 'summer', the season of highest water temperature, falls in July, August or September in the English Channel. Similarly, the depth of 'winter', the season of lowest temperature, occurs in January, February, March or April.

Fig. 3 shows for each year the maximal monthly mean temperature for three districts of the English Channel, at the mouth, in the centre, and towards the Straits of Dover.[1] The rings immediately below the upper scale of years indicate the years in which the octopus was recorded as notably abundant. We see that, as far as sea temperature is concerned, the summer of 1893 (as judged by the highest monthly mean temperature) was indeed warm, at least in the western part of the Channel, but it was followed immediately by a distinctly cool summer. Thereafter succeeding summers became gradually warmer, culminating in an outstandingly hot one in 1899. Turning to the 1950 plague, we observe that although the summer of 1949 was very hot, in

[1] The figures for the western district for the years after 1904 are based on the data available for the Regions 5, 8, 9 and 10 used by Lumby (1935); for 1904 and earlier years they are based on observations at the *Seven Stones* lightship alone (see E. C. Jee, 1919). Similarly, the central district comprises Regions 17, 18, 21, 22, 23, 24 and 25; and the eastern district, Regions 30, 31, 32, 33, 35, 38 and 40.

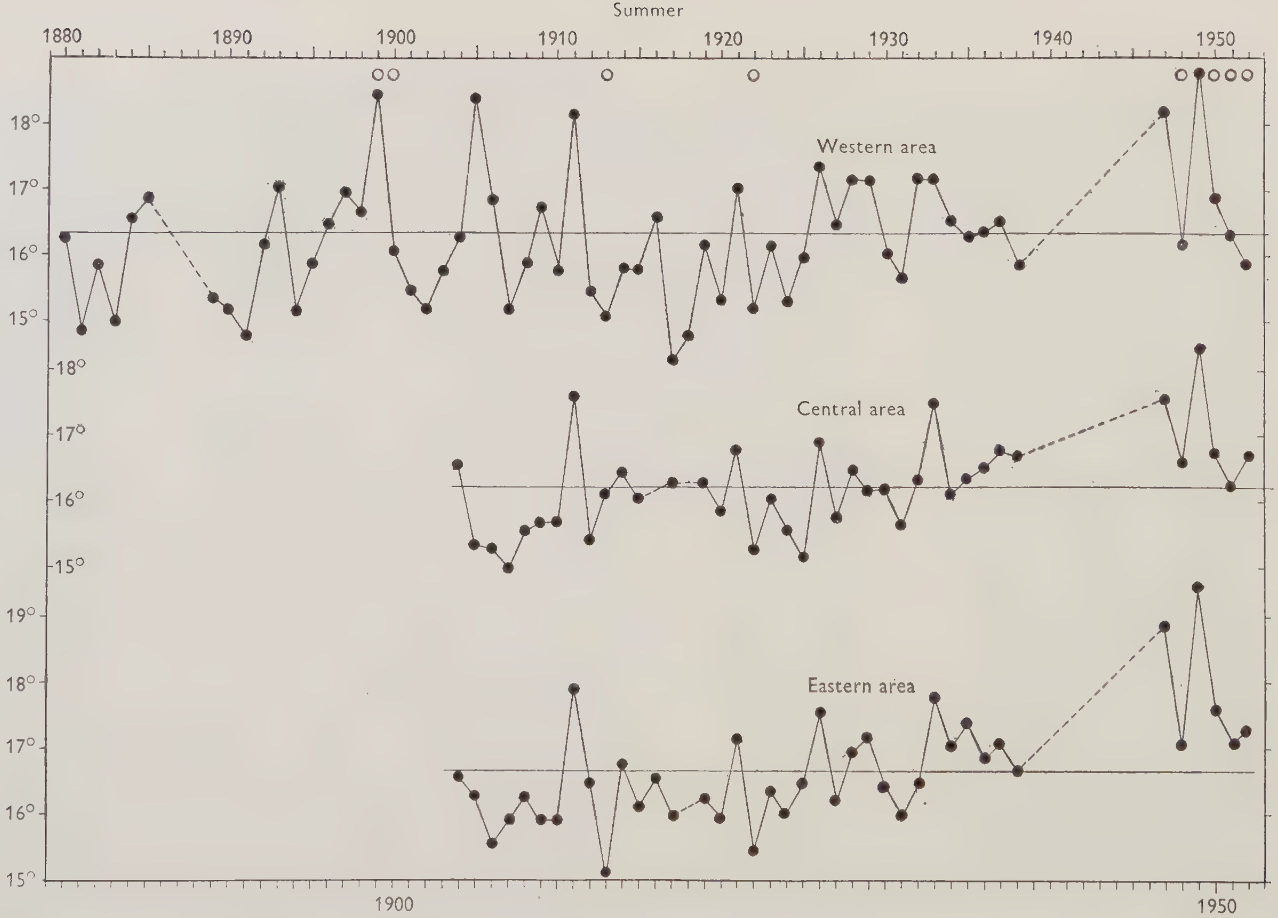

Fig. 3. The *maximal* monthly mean surface water temperature (° C) for each year in three districts of the English Channel. The western area comprises Regions 5, 8, 9 and 10 (see Lumby, 1935); the central area, Regions 17, 18, 21, 22, 23, 24 and 25; and the eastern area, Regions 30, 31, 32, 33, 35, 38 and 40. For the years before 1905, the figures for the western area are based on observations at the Seven Stones lightship only. The rings beneath the scale of years indicate years in which *Octopus* was

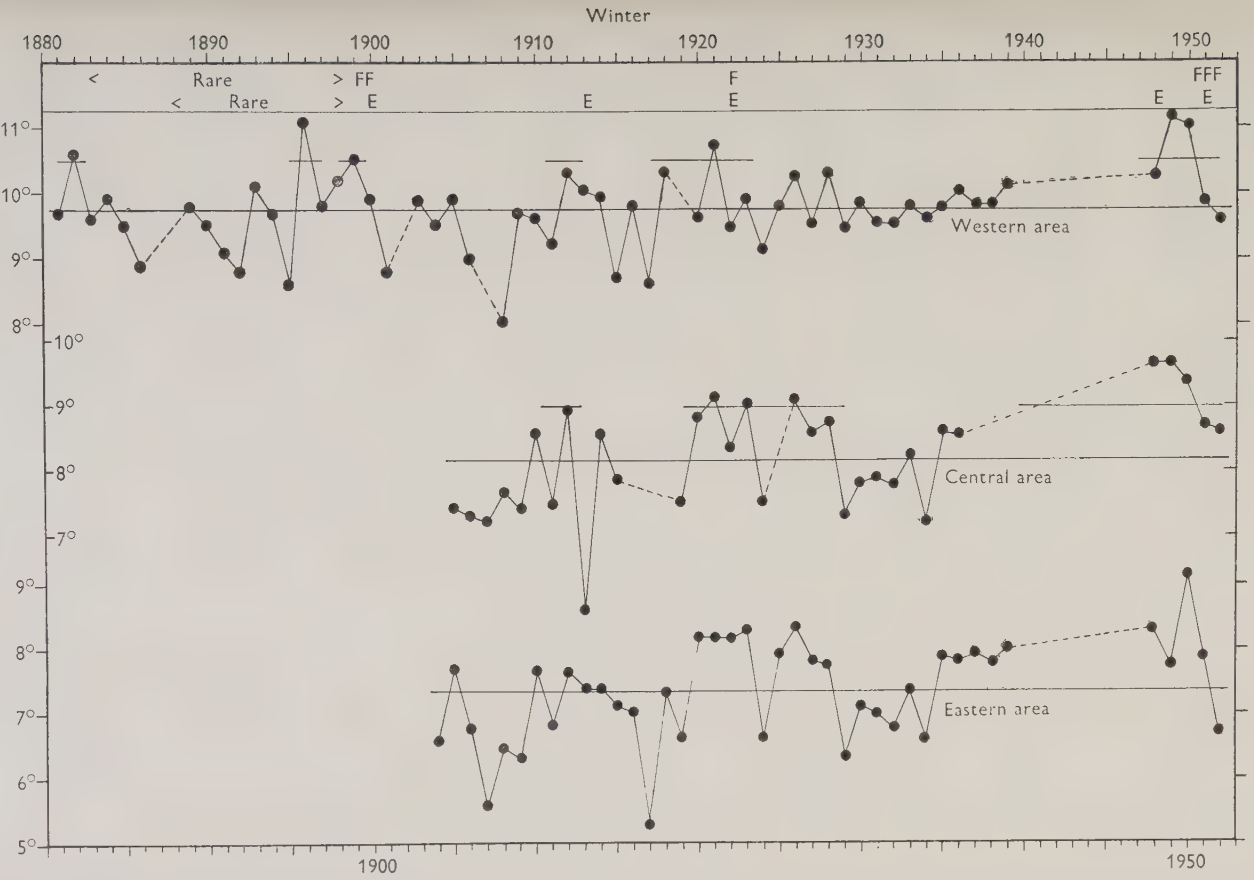

Fig. 4. The *minimal* monthly mean surface water temperature (° C) for each year in three districts of the English Channel. For details, see legend to Fig. 3. For the western area, the figures for the years before 1906 are based on observations at the Seven Stones lightship only. The lettering beneath the scale of years indicates the years in which *Octopus* was reported as notably abundant on the French coast (F), and on the English coast (E).

all parts of the Channel, the numbers of octopus do not appear to have reached plague proportions anywhere until the following year. The outbreak in 1913 appears to have taken place in a year with a cool summer, though that of 1911 was very hot. The summer of 1922 was also cool, following a fairly warm one in 1921. Similarly, the cool summer of 1948 follows a hot one in 1947. There are, however, other summers which may be classified as well above normal, even though not outstandingly hot—for example 1926 and 1933—which do not appear to have been followed by notable increases in the octopus population. It would seem that, on the whole, the association, if any, between numbers of octopus and summer temperature is not close.

Turning to the winter conditions, shown similarly by the minimal monthly mean temperature for each year (Fig. 4), we may see a somewhat clearer connexion. Outbreaks of *Octopus* can be seen to have occurred either in the same year as, or in the year immediately after, the winter temperatures were outstandingly high in both western and central regions at the same time; whenever, that is, the winter temperature (as given by the monthly mean) has not fallen below about $10\frac{1}{2}°$ C at the mouth of the Channel, nor below 9° C in the central district. Conditions in the years 1912 and 1948 appear to fall a little short according to this criterion. In addition, with the possible exception of 1896, there were no outstandingly warm winters in the two regions at the same time which were not soon followed by increases in the abundance of *Octopus*. The evidence for the association to which attention is drawn here is not claimed as sufficient for complete acceptance, but it seems likely that conditions in winter influence the behaviour of the octopus more directly, or to a greater extent, than those of summer.

Notable increases in the numbers of octopus in the inshore waters appear to follow very closely on the heels of a warm winter. A period of several warm years in which the octopus population builds up under improved conditions of reproduction is evidently not a pre-requisite to a plague, as Garstang suggested. The inference is, rather, that the initial increase in numbers springs in the first instance from immigration of greater numbers of adults into inshore waters, and perhaps even into the Channel. Thereafter, since spawning is likely to be on an increased scale (in consequence of the greater numbers of adults present) and if the rate of survival in the larvae is not unduly diminished by particularly unfavourable conditions, the plague may be intensified by these means in subsequent years.

It seems that the full development of a plague occurs a year later on the English side than on the French. The reports give the impression that in both 1899 and 1950, when numbers on the French coast had already become very high, they were only in the rising stage on the English side. We may agree with Garstang (1900, pp. 270–1) in thinking that the explanation for this lies in the pressure of population on the French side becoming so great as to drive the animals to emigrate to the English coasts. It is doubtful whether water temperature plays any part in this, since, although the summer of 1899 was exceedingly hot, that of 1950 was only slightly in excess of the average. In 1949, when the summer temperature was even higher than in 1899, the plague had not yet developed on the French side, so far as the records show.

Lowest Tolerable Temperature and Migration

As a rule the octopus appear to move away from the inshore regions when the height of summer has passed, and to spend the winter in the deeper waters offshore. After the winter, they return inshore for spawning. There is some slight indication that shoreward movement is discouraged by very severe winters, not only in the spring immediately following, but for a period of a year or more. The severity of the winter of 1895, for instance, may account for the absence of an outbreak of *Octopus* after the very mild winter of 1896. The winter of 1947 was extremely cold; the monthly mean temperature for February 1947 was 4·8° C at Jersey and 4·9° C off Plymouth. This may have delayed the outbreak of the plague, perhaps, but the evidence for this point is too indefinite to allow reliable conclusions to be drawn.

On the other hand, in some years octopus are known to remain inshore throughout the winter. An occasional juvenile octopus was caught off Plymouth by the research vessels of the Marine Biological Association in March 1950, and throughout the following winter (1951) *Octopus* were being caught by the *Sabella* within 16 miles of Plymouth Breakwater (see Appendix). During March 1950 the sea temperature near Plymouth Breakwater (Station L. 2) was between 9 and 10° C, and about ½° C higher near the Eddystone (St. E. 1). In winter 1951 the sea temperature at St. L. 2 fell to 8° C, possibly a little below, and to about 8½° C near the Eddystone during this period. The average winter temperature—the monthly mean for February—in Region 11 (see Lumby, 1935) is estimated at 8·7° C for the period 1903–27 and 8·2° C for the period 1928–51 (these latter figures are not yet published). On the other side of the Channel, as Mr Baal informs us, *Octopus* remained inshore at Jersey during three successive winters, those of 1950, 1951 and 1952. They did not leave these waters until October 1952. The lowest monthly mean temperatures in these waters were 8½°, 6° and 7½° C, for these three winters respectively. These compare with an average value of 7° C over the years 1933–39, 1946–52. In October 1952, when the animal left these waters, the average temperature was 13½° C; in November, 10½° C.

From this we may conclude with assurance that *Octopus* tolerates temperatures as low as 6° C. However, in view of the fact that this value is well below the average winter temperature of the water both off Plymouth and at Jersey, and since the animal is not normally present in these waters in winter, it must be concluded that the occurrence of *Octopus* at this season is governed by temperature only to quite a limited extent. It is clearly not true that the animal may always be expected to be present when the water temperature is above 6° C, and there is thus no justification for inferring from the absence of *Octopus* from waters of a certain temperature that the animal is intolerant of that temperature. We therefore cannot yet determine from the evidence before us, what is the lowest temperature *Octopus* can tolerate; for a warm-water animal, however, it is evidently surprisingly low.

At the mouth of the English Channel the lowest monthly mean temperature recorded, that of 1908, was just above 8° C (Fig. 4). Thus only very infrequently indeed would the octopus find a water temperature below 6° C in this part of the English Channel. Moreover, as the chart of average winter temperature shows (Fig. 5), the octopus can find, on the average, water of a tolerable temperature almost everywhere in the English Channel. In view of this, and also of the apparent ability of the animal to increase its numbers inshore very quickly after a warm winter, it would not be surprising to find that there is almost always quite a large population of *Octopus* inhabiting the Channel, which is not noticed except in some special seasons.

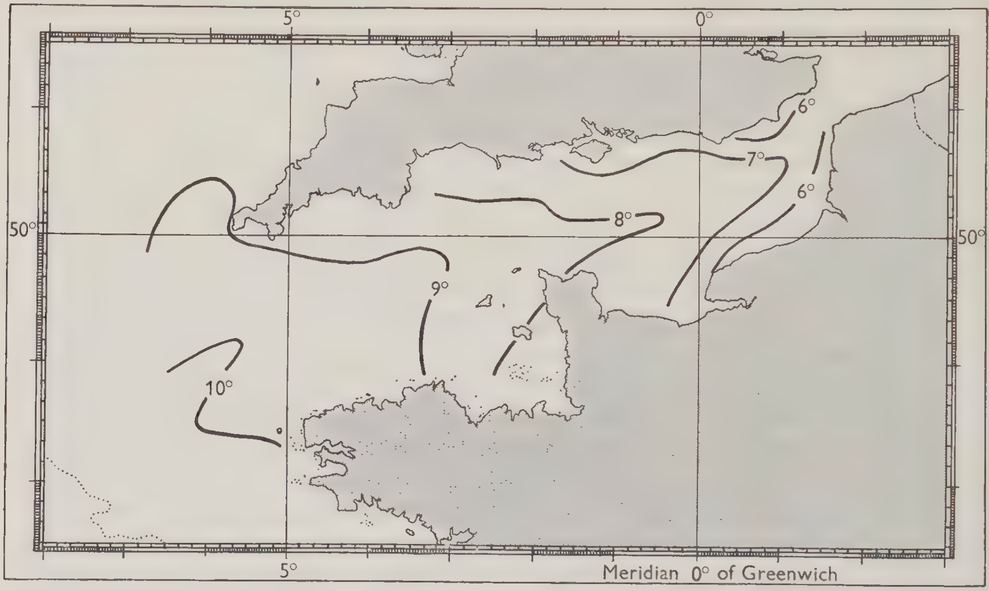

Fig. 5. The *minimal* monthly mean temperature (° C) of the surface water in the English Channel, based on observations over the period 1903–27.

Discussion

The disturbances caused by the abundance of *Octopus* in 1950–51 followed the same general pattern as in 1899–1900. Recently, as in the earlier 'plague', shell-fishing had to be temporarily abandoned at some points on the Devon coast and on the French coast. In so far as it has been possible to get authentic records for 1950–51, most of the octopus were of medium size with an arm span of 2½–3 ft., but specimens reaching 5–5½ ft. were reported. They were therefore smaller on the average than those mentioned by Garstang (1900).

We believe that the distribution of larvae shown in Fig. 1 gives conclusive evidence that the main breeding grounds of *Octopus* in the south-western Channel are in coastal waters, from Ushant eastwards along the north coast of France, and around the Channel Islands, to as far east as the neighbourhood of Grandcamp-les-Bains. Occasional spawning on the English side appears to be insufficiently frequent to be represented in the catches.

We have not been able to add much to greatly needed information on the growth of this species. It appears, however, that larvae hatched the previous year reach a size at least 14 mm in mantle length by March of the following year (see p. 515). This particular specimen may have hatched very late in the breeding season and may have lived through the winter without much growth. In size it may be comparable with young octopus of very small size reported by Devon fishermen in the autumn of 1950. From scattered reports received from the whole south coast most of the octopus seen in July and August 1950 were about 2½ ft. in arm span, and, on this point, we think it extremely likely that they were not more than two years old, and although we have no evidence on this point, some may have been only just over a year old.

During the recent years of numerical abundance of *Octopus* this species has been present in inshore waters the whole year round on the English coast and in the Channel Islands. Even in Jersey, as Mr H. J. Baal informs us (*in litt.*), in normal years *Octopus* disappears from inshore waters in winter but since the winter of 1949–50 it has been quite common throughout the period but disappeared into deeper water in October 1952 (Baal, 1953). Sinel (1906, p. 215) implies that it is present only in summer at Jersey in normal years in the words: 'it is constant during the summer months'. In another paragraph (p. 222) dealing with the capture of *Octopus*, he indicates his belief in a southerly migration of *Octopus* in the autumn: 'Yet another plan, much used in the autumn on the north coast of Jersey: in this particular locality, and at this time, they sometimes swarm on the sea surface, swimming in the method last described, no doubt to some more congenial shore. Men armed with long bamboo rods, with large hooks at the end, station themselves on outlying rocks, and simply hook them out as they pass. I have seen many tons' weight caught in one locality by this method, and being used to manure the land.' Mr Baal also informs us that he has seen this southerly movement at this place in the autumn but there is as yet no real evidence of large-scale migratory movement other than the migration across the Channel in plague years and seasonal retreat into deeper water (see p. 516). In *Eledone* these seasonal movements, from offshore fishing grounds to inshore waters, which take place in the summer, followed by a retreat to deeper water in winter, have been reported by Isgrove (1909). *Eledone cirrhosa* is much rarer on the south side of the Channel than in English waters, but after the octopus became more abundant, *Eledone* became scarcer, presumably because it is eaten by the larger species.

The virtual disappearance of the lobster (*Homarus vulgaris*) on offshore fishing grounds with the arrival of *Octopus* (see Appendix I) was to be expected. The increase in numbers of crawfish (*Palinurus vulgaris*) on the trawling grounds implies that they were being driven out of their usual haunts to areas where *Octopus* was less abundant. There is no information available to suggest that the spider crab (*Maia squinado*) was being driven ashore on the English coast as in 1899–1900. Baal (1953), however, notes that in the autumn of 1952 spider crabs were numerous in shallow lagoons exposed at spring tides in Jersey. He observed that 'the spider crabs had collected into large heaps, about two feet and three feet in diameter, with their legs so entangled as to make it difficult to separate a crab from the heap. The octopuses captured some from the outside of the masses but the greater number survived, and day after day the heaps remained.' As Baal implies, this curious behaviour may have a survival value for the spider crab.

We wish to thank the Director of Fishery Investigations, Lowestoft, for readily placing the plankton samples of the *Sir Lancelot* cruises at our disposal. We are also greatly indebted to Mr H. J. Baal, Lieut.-Commander W. H. Batten, Lieut.-Commander C. A. Hoodless, D.S.C., and Prof. J. Z. Young, F.R.S., for information on local abundance of octopus.

Thanks are also due to Dr H. W. Parker (for larvae collected by him off the Casquets in 1950), Mr F. S. Russell, F.R.S. (for a juvenile octopus from Plymouth), and Alderman W. P. D. Stebbing for an adult octopus from Deal.

SUMMARY

The great abundance of *Octopus vulgaris* on the English coast of the Channel in 1950–51 recalls the previous 'plague' in 1899–1900 when, as now, Devon and Cornwall seemed affected the most.

It has been possible to demonstrate from plankton surveys of the Channel in 1948, 1949 and 1950 that planktonic larvae of *Octopus* originate on the south side of the Channel in the area between the Cotentin peninsula, the Channel Islands, and Ushant (Fig. 1). There is no indication of substantial breeding on the English coast.

Spawning commences in May in the extreme south-west, but the main spawning occurs in June and July, larvae becoming numerous in July and August. Larvae still planktonic in September are in process of dispersal to the north and east. The water temperature when spawning begins is estimated to be about $11\frac{1}{2}$–12° C.

Apart from a suggestion that mild winter conditions encourage the species

to become very abundant almost immediately on the French coast, *Octopus* appears to be influenced very little by sea temperature. Dearth of food seems to be the cause of subsequent trans-Channel migration of adults. From the evidence available *Octopus* tolerates temperatures as low as 6° C.

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APPENDIX I

By Lieut.-Commander C. A. HOODLESS, D.S.C., R.N.R.,
Master, R.V. *Sabella*

Records of *Octopus vulgaris* and *Eledone cirrhosa* trawled off Plymouth between 1 June 1948 and 30 June 1951 by R.V. *Sabella*

All the trawl hauls were made within a 16-mile radius of Plymouth Breakwater with a standard otter trawl with a head line of 56 ft.

1948. In the 7 months of this year covered by the record 2 *Eledone* were taken, both in October.

1949. 1 *Eledone* taken in October.

1950. Up to the commencement of the accompanying table single specimens of *Eledone* were taken in January, July and August.

During the whole of the above periods *Sabella* was making an average of 11 trawl hauls a month.

The first record of *Octopus* was on 4 September 1950. From that date *Homarus vulgaris* almost completely disappeared from the trawling grounds but *Palinurus vulgaris* was taken in much larger numbers than before.

The table below shows the numbers of *Octopus* and *Eledone* taken between September 1950 and June 1951, with the number of trawl hauls and the average number of specimens per haul:

		Eledone cirrhosa		*Octopus vulgaris*	
Month	No. of trawl hauls	No. of specimens	Average per haul	No. of specimens	Average per haul
September 1950	20	1	0·05	2	0·1
October	15	—	—	3	0·2
November	16	—	—	2	0·1
December	9	—	—	9	1·0
January 1951	13	3	0·2	3	0·2
February	13	—	—	29	2·2
March	3	—	—	4	1·3
April	16	1	0·1	13	0·8
May	20	—	—	17	0·9
June	10	—	—	2	0·2

J. Mar. biol. Ass. U.K. (1954) 33, 537–560
Printed in Great Britain

A PRELIMINARY CHECK-LIST OF BRITISH MARINE DIATOMS

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The present list of diatoms may be considered an extension of the recent check-list of British marine algae by Parke (1953). It includes not only truly marine species, both sessile and planktonic, but also species inhabiting brackish waters with a few fresh-water species that manage to live, though somewhat precariously, in estuarine conditions.

It has been compiled for the following reasons: (1) to indicate the extent of the diatom flora around British coasts, so that comparisons may be made with that of the other countries; (2) to assist workers in the identification of material by indicating the size of any genus known to inhabit our shores; (3) to check the arrival of new entrants to the British flora; and (4) to provide a basis for a more detailed work, now in preparation. The check-list does not presume to lay down finalized decisions on the taxonomic rank ascribed to any organism or to consider the criteria upon which such ranks are based.

The vexed question of species, varieties and forms and the characters appertaining to them, as well as the degree to which such characters are possessed, is no part of the work at its present stage. Most of the varieties and forms cited are used in the accepted sense, and exceptions are explained in the notes.

As before, genera are listed alphabetically under the family, and species alphabetically under the genus. Synonyms are in brackets, and notes are appended at the end of each suborder: the number in brackets following a name in the list refers to these notes.

Planktonic species are marked with an asterisk.

Diatoms are considered as a Class of Algae, BACILLARIOPHYCEAE, comprising one order, BACILLARIALES, which is divided into ten suborders (Hendey, 1937, 1951), as follows:

1. Discineae
2. Aulacodiscineae
3. Auliscineae
4. Biddulphiineae
5. Soleniineae
6. Araphidineae
7. Raphidioidineae
8. Monoraphidineae
9. Biraphidineae
10. Surirellineae

The flora consists of 771 species arranged in 104 genera. The list includes all marine and brackish-water species that have been *recorded*, with the omission of some that are doubtful or imperfectly described. It has not been possible to check all those listed.

The compiler will be grateful to hear of any errors or omissions, inevitable in a preliminary survey such as this, as well as to receive material or mounted slides that may help in advancing knowledge of our diatom flora.

Finally, it is hoped that this list will provide a stimulus for field-work of which a vast amount is yet necessary.

BACILLARIOPHYCEAE

BACILLARIALES

Suborder I. DISCINEAE

Coscinodiscaceae

- ACTINOCYCLUS Ehrenberg, 1838. (1)
  - *fulvus* (W. Sm.) Ralfs ex Pritch.* (2)
    - (*Eupodiscus fulvus* W. Sm.)
  - *octonarius* Ehrenb.*
    - (*A. ehrenbergii* Ralfs ex Pritch.)
    - var. *octonarius*
    - var. *crassus* (W. Sm.)*
      - (*A. crassus* (W. Sm.) Ralfs ex Pritch.)
      - (*A. ehrenbergii* var. *crassus* (W. Sm.) Hust.)
      - (*Eupodiscus crassus* W. Sm.)
    - var. *ralfsii* (W. Sm.)*
      - (*A. ehrenbergii* var. *ralfsii* (W. Sm.) Hust.)
      - (*Eupodiscus ralfsii* W. Sm.)
      - (*Coscinodiscus fuscus* Norm.)
    - var. *sparsus* (Greg.)*
      - (*A. sparsus* (Greg.) Rattray)
      - (*Eupodiscus sparsus* Greg.)
    - var. *tenella* (de Bréb.)*
      - (*A. ehrenbergii* var. *tenella* (de Bréb.) Hust.)
      - (*A. moniliformis* Ralfs ex Pritch.)
      - (*Eupodiscus tenellus* de Bréb.)
  - *roperii* (de Bréb.) Grun. ex Van Heurck*
    - (*ovalis* Grun.)
    - (*Eupodiscus roperii* de Bréb.)
  - *subtilis* (Greg.) Ralfs ex Pritch.*
    - (*falsus* W. Sm. ?)
    - (*subtilis* var. *disjuncta* Ratt.)
    - (*Eupodiscus subtilis* Greg.)
    - (*E. gregoryanus* de Bréb.)
- COSCINODISCUS Ehrenberg, 1838.
  - *anguste-lineatus* A. Schm.*
  - *asteromphalus* Ehrenb.*
    - var. *asteromphalus*
    - var. *hybrida* Grun.*
  - *centralis* Ehrenb.* (3)
  - *concinnus* W. Sm.*
  - *curvatulus* Grun. ex Schm.*
  - *divisus* Grun.
  - *excentricus* Ehrenb.*
  - *fimbriatus* Ehrenb.*
  - *gigas* Ehrenb.*
  - *grani* Gough*
  - *joergensenii* Ostenf.*
  - *jonesianus* (Grev.) Ostenf.*
    - var. *jonesianus*
    - var. *commutatus* (Grun.) Hust.*
  - *kutzingii* A. Schm.*
  - *leptopus* Grun. ex Van Heurck*
  - *lineatus* Ehrenb.*
  - *marginatus* Ehrenb.*
  - *nitidus* Greg.*
  - *nodulifera* Jan. ex Schm.*
  - *obscurus* A. Schm.*
  - *oculus-iridis* Ehrenb.*
  - *perforatus* Ehrenb.*
    - var. *perforatus*
    - var. *pavillardi* (Forti) Hust.*
  - *radiatus* Ehrenb.*
  - *rothii* (Ehrenb.) Grun.*
    - var. *rothii*
    - var. *normanii* (Greg.) Grun.*
      - (*C. subtilis* var. *normanii* Van Heurck)
      - (*C. normanii* (Greg.) Brockm.)
  - *stellaris* Roper*
  - *sub-bulliens* Jörg.* (3)
  - *subtilis* Ehrenb.*
  - *woodwardii* Eulenst.* (4)
- COSCINOSIRA Gran, 1900. (5)
  - *oestrupii* Ostenf.*
  - *polychorda* Gran*
- CYCLOTELLA (Kützing) de Brébisson, 1838.
  - *kutzingiana* Thwaites (6)
  - *striata* (Kütz.) Grun.
- DRURIDGEA Donkin, 1861.
  - *compressa* (West) Donk.*
    - (*geminata* Donk.)
    - (*Podosira compressa* West)
- ENDICTYA Ehrenberg, 1845.
  - *oceanica* Ehrenb.*
- HYALODISCUS Ehrenberg, 1845. (7)
  - *radiatus* (O'Meara) Grun.*
    - (*Pyxidicula radiata* O'Meara)
  - *scoticus* (Kütz.) Grun.*
    - (*Cyclotella scotica* Kütz.)
    - (*Podosira smithiana* Grun.)
  - *subtilis* Bail.*
    - (*franklini* (Ehrenb.) Cleve)
    - (*Podosira subtilis* Mann)
    - (*Craspedodiscus franklini* Ehrenb.)

MELOSIRA Agardh, 1824.
arctica (Ehrenb.) Dick.
(*hyperborea* Schütt)
jurgensii Agardh
moniliformis (O. F. Müll.) Agardh
(*borreri* Grev. in Hook.)
nummuloides (Dillw.) Agardh
westii W. Sm.
PARALIA Heiberg, 1863. (8)
sulcata (Ehrenb.) Cleve
(*marina* Heib.)
(*Gaillionella sulcata* Ehrenb.)
(*Orthosira marina* W. Sm.)
var. *sulcata*
f. coronata (Ehrenb.) Grun.
f. radiata Grun.
var. *biseriata* Grun.
PHACODISCUS Meunier, 1910.
punctulatus (Greg.) Meun.*
(*Coscinodiscus punctulatus* Greg.)
PLANKTONIELLA Schütt, 1893.
sol (Wall.) Schütt*
(*woltereckі* Schimp.)
(*Coscinodiscus sol* Wall.)
PODOSIRA Ehrenberg, 1840. (9)
montagnei Kütz.*
stelliger (Bail.) Mann*
(*maculata* W. Sm.)
(*Hyalodiscus stelliger* Bail.)
(*Melosira maculata* Lagerst.)
POROSIRA Jörgensen in Nordgaard, 1905.
glacialis (Gran) Jörg.* (10)
(*Podosira glacialis* Cleve)
(*P. hormoides* var. *glacialis* Grun.)
(*Lauderia glacialis* (Grun.) Gran)
PYXIDICULA Ehrenberg, 1833.
cruciata Ehrenb.* (11)
ROPERIA Grunow ex Van Heurck, 1885. (12)
tessellata (Roper) Grun.*
(*Eupodiscus tessellatus* Roper)
(*Actinocyclus tessellatus* (Roper) Ralfs ex Pritch.)
SKELETONEMA Greville, 1865.
costatum (Grev.) Cleve*
(*Melosira costata* Grev.)
mirabile Grun. ex Van Heurck*
STEPHANODISCUS Ehrenberg, 1845.
astraea (Ehrenb.) Grun.* (13)
dubius (Fricke) Hust.*
hantzschii Grun.*
STEPHANOPYXIS Ehrenberg, 1844.
palmeriana (Grev.) Grun.*
turris (Grev.) Ralfs ex Pritch.*
THALASSIOSIRA Cleve, 1873. (5)
baltica (Grun.) Ostenf.*
bioculata (Grun.) Ostenf.*
condensata Cleve*
decipiens (Grun.) Jörg.*
fallax Meun.*
fluviatilis Hust.*
gravida Cleve*
hyalina (Grun.) Gran*
nordenskioldii Cleve*
rotula Meun.*
subtilis (Ostenf.) Gran*

Hemidiscaceae

HEMIDISCUS Wallich, 1860. (14)
cuneiformis Wall.*
(*Euodia inornata* Castr.)
(*E. cuneiformis* Schütt)

Actinodiscaceae

ACTINOPTYCHUS Ehrenberg, 1843.
amblyoceros (Ehrenb.) Schm.*
senarius Ehrenb.*
(*undulatus* Bail.)
splendens (Shad.) Ralfs ex Pritch.*
ARACHNOIDISCUS Deane ex Pritchard, 1852. (15)
ehrenbergii Bail. ex Ehrenb. (16)
ASTEROMPHALUS Ehrenberg, 1844.
flabellatus (de Bréb.) Grev.*
heptactis (de Bréb.) Ralfs ex Pritch.*
hookerii Ehrenb.*

Notes on Discineae

(1) *Actinocylcus* Ehrenb. is placed in the Coscinodiscaceae (Hendey, 1937) because the general valve structure resembles that of *Coscinodiscus* Ehrenb. and the presence of a marginal ocellus is regarded as of secondary importance. The genus is obviously related to *Roperia* Grun. and to the Eupodiscaceae, where it is placed by Hustedt and Cleve-Euler, but in that position it assumes affinities with *Auliscus* Ehrenb. which stretches relationships beyond reasonable bounds.

(2) Probably a form of *Actinocyclus octonarius* or intermediate between it and *A. subtilis* (Greg.) Ralfs ex Pritch.

(3) Cleve-Euler (1951) makes this a variety of *Coscinodiscus asteromphalus* Ehrenb.

(4) Rattray makes this a synonym of *C. apiculatus* Ehrenb., but Mann (1907) points out that it is nearer to *C. radiatus* Ehrenb. Hustedt unites it with *C. perforatus* Ehrenb.

(5) Cleve-Euler (1951) makes this a subgenus of *Coscinodiscus* Ehrenb. This is not in the least useful, as *Coscinosira* Gran and *Thalassiosira* Cleve have characteristic colony formation and are genera well recognized by plankton biologists, who make most of their determinations upon either living or formalin-preserved material.

(6) Cleve-Euler (1951) credits this combination to Chauvin.

(7) Hustedt's classification is followed here in that *Hyalodiscus* is reserved for those species that possess an umbilical line at about half the valve radius. More work is required on British material.

(8) *Paralia* Heiberg is separated from *Melosira* on account of the punctate-areolate structure of the valve margin and mantle. Lebour (1930), Gran (1905), and Cleve-Euler (1951) accept *Paralia*; Hustedt gives it subgeneric rank. Often found in the plankton, particularly after winter storms.

(9) Cleve-Euler (1951) makes *Podosira* Ehrenb. a subgenus of *Melosira* Agardh.

(10) Gran (1905) and Lebour (1930) place this species in *Lauderia*. Hustedt separates them on account of the spinulae, which cover the valve in *Porosira* but are marginal only in *Lauderia*.

(11) A very doubtful species. Three species only are known from European waters, and more work is required on this group.

(12) *Roperia* Grunow connects *Actinocyclus* Ehrenb. with the Eupodiscaceae. Some authorities place it there, but it has closer affinities with *Coscinodiscus* Ehrenb.

(13) Normally considered as a fresh-water species, but is often found living in marine conditions.

(14) Very variable in outline and size, with a world-wide distribution.

(15) The generic name *Arachnoidiscus* Deane was accepted for conservation against *Hemiptychus* Ehrenb., *Ber. Akad. Wiss., Berl.*, 1848, p. 7, and *Arachnodiscus* J. W. Bailey ex Ehrenb., *Ber. Akad. Wiss., Berl.*, 1849, p. 63, by the Special Committee for Diatomaceae at the 7th International Botanical Congress, Stockholm, July 1950 (*Int. Code bot. nomencl.*, 1952, p. 70, Utrecht).

(16) This record is doubtful. Two records are given in the literature of the nineteenth century, both for the west coast. The species has been recorded (unpublished) from the Thames Estuary; but it would be unwise to consider the species as other than an alien in the British flora. With such a characteristic species there can be no case for misidentification, so it can be assumed only that the species was carried to our shores mechanically. The species is common on the Californian coast and around Cape Town.

Suborder 2. AULACODISCINEAE

Eupodiscaceae
AULACODISCUS Ehrenberg, 1844. (1)
argus (Ehrenb.) Schm.*
(*Eupodiscus argus* (Ehrenb.) W. Sm.)
sollitianus Norman*

EUPODISCUS Rattray, 1888. (2)
radiatus Bail.*
(*Aulacodiscus radiatus* (Bail.) Brightw.)

Notes on Aulacodiscineae

(1) *Aulacodiscus* Ehrenb. (*Ber. Akad. Wiss., Berl.*, 1844, p. 73) based on *A. crux* Ehrenb. was accepted for conservation by the Special Committee for Diatomaceae at the 7th International Botanical Congress, Stockholm, July 1950 (*Int. Code bot. nomencl.*, 1952, p. 70, Utrecht) against *Tripodiscus* Ehrenb., *Tetrapodiscus* Ehrenb., *Pentapodiscus* Ehrenb. and *Eupodiscus* Ehrenb.

(2) The name *Eupodiscus* was accepted for conservation in the sense that Rattray used it (*J.R. micr. Soc.*, 1888, p. 909), i.e. for *E. radiatus* Bail. (*Int. Code bot. nomencl.*, 1952, p. 71)—see note (1).

Suborder 3. AULISCINEAE

Auliscaceae
AULISCUS Ehrenberg, 1843.
sculptus (W. Sm.) Ralfs ex Pritch. (1)

Note on Auliscineae

(1) This species occurs but infrequently around British coasts, and it is thought that a more intensive search, particularly along the south and west coasts, would reveal a more general distribution of it, also that *Auliscus caelatus* Bail., a species favouring warmer water, might be encountered. *A. caelatus* is considered by some authorities to be identical with *A. sculptus*. Further researches on this species are required.

Suborder 4. BIDDULPHIINEAE

Biddulphiaceae (1)
Bellerochea Van Heurck, 1885.
malleus (Brightw.) Van Heurck*
(_Triceratium malleus_ Brightw.)
Biddulphia Gray, 1821. (2)
alternans (Bail.) Van Heurck
(_Triceratium alternans_ Bail.)
antediluviana (Ehrenb.) Van Heurck
(_vesiculosa_ (Agardh) Boyer)
(_Amphitetras antediluviana_ Ehrenb.)
aurita (Lyngb.) de Bréb.* (3)
var. _aurita_
var. _obtusa_ (Kütz.) Hust.*
(_B. obtusa_ (Kütz.) Ralfs ex Pritch.)
(_B. roperiana_ Grev.)
(_Odontella obtusa_ Kütz.)
granulata Roper*
laevis Ehrenb.*
mobiliensis (Bail.) Grun. ex Van Heurck*
(_baileyi_ W. Sm.)
(_Zygoceros mobiliensis_ Bail.)
pulchella Gray
(_biddulphiana_ (Smith) Boyer)
regia (Schultze) Ostenf.*
rhombus (Ehrenb.) W. Sm.*
f. _rhombus_
(_Zygoceros rhombus_ Ehrenb.)
f. _trigona_ Hust.*
sinensis Grev.* (4)
spinosa (Bail.) Boyer* (5)
(_Triceratium spinosum_ Bail.)
(_T. armatum_ Roper)
(_T. serratum_ Wall.)
(_T. setigerum_ Bail.)
striolata (Ehrenb.)* (6)
(_Triceratium striolatum_ Ehrenb.)
(_Trigonium striolatum_ (Ehrenb.) Mann)
Cerataulina Peragallo & Schütt, 1896.
pelagica (Cleve) Hendey*
(_bergonii_ (Perag.) Schütt)
Cerataulus Ehrenberg, 1843 (1844). (7)
smithii Ralfs ex Pritch.*
(_Biddulphia smithii_ Van Heurck)
turgidus Ehrenb.
Ditylum L. W. Bailey, 1862.
brightwellii (West) Grun. ex Van Heurck*
(_trigonum_ Bail.)
(_undulatum_ (Brightw.) Mann)
(_Triceratium brightwellii_ West)
(_T. undulatum_ Brightw. e.p.)
Eucampia Ehrenberg, 1840.
zoodiacus Ehrenb.*
(_groenlandica_ Cleve) (8)
(_britannica_ W. Sm.)
(_nodosa_ Schm.)
Hemiaulus Ehrenberg, 1844.
hauckii Grun. ex Van Heurck*
(_delicatulus_ Lemm.)
Isthmia Agardh, 1832.
enervis Ehrenb.
(_obliquata_ Agardh e.p.)
(_Isthmiella enervis_ Cleve) (9)
var. _enervis_
var. _subcylindrica_ Taylor
nervosa Kütz.
(_obliquata_ Ehrenb.)
(_obliquata_ Agardh e.p.)
Lithodesmium Ehrenberg, 1840.
undulatum Ehrenb.*
(_victoriae_ Karsten)
(_Triceratium undulatum_ Brightw.)
(_T. intricatum_ West)
(_Ditylum intricatum_ Grun.)
(_D. undulatum_ Mann.)
Streptotheca Shrubsole, 1890.
thamesis Shrubs.*
Triceratium Ehrenberg, 1840.
favus Ehrenb.* (10)
(_muricatum_ Brightw.)
(_fimbriatum_ Wall.)
(_ferox_ Castr.)
(_sarcophagos_ Castr.)
(_Biddulphia favus_ (Ehrenb.) Van Heurck)
Trigonium Cleve, 1868.
arcticum (Brightw.) Cleve (11)
(_Triceratium arcticum_ Brightw.)

Chaetoceraceae
Chaetoceros Ehrenberg, 1844. (12)
adhaerans Mangin*
affine Laud.*
(_schuttii_ Cleve in Gran)
var. _affine_
var. _willei_ (Grun.) Hust.*
armatum West*
atlanticum Cleve*
boreale Bail.*
breve Schütt*
ceratosporum Ostenf.*
(_gracilis_ Apstein)
cinctus Gran*
coarctatum Laud.*
compressum Laud.*
(_contortum_ Schütt)
concavicorne Mangin*
(_criophilus_ Gran)
constrictum Gran*
convexicorne Mangin*
(_peruvianus_ Gran)
convolutum Castr.*
coronatum Gran*
crinitus Schütt*
curvisetum Cleve*
danicum Cleve*
debile Cleve*
decipiens Cleve*
densum Cleve*
didymum Ehrenb.*
difficile Cleve*
diversum Cleve*
eibenii (Grun.) Meun.*

CHAETOCEROS (*cont.*)
exospermum Meun.★
externum Gran★
furca Cleve★
glandazi Mangin★
gracile Schütt★
holsaticum Schütt★
(*balticus* Cleve)
imbricatum Mangin★
ingolfianum Ostenf. in Gran★
janischianum Castr.★
(*dichaeta* Ehrenb.)
laciniosum Schütt★
lauderi Ralfs in Laud.★
(*weissflogii* Schütt)
lorenzianum Grun.★
mitra (Bail.) Cleve★
(*Dicladia mitra* Bail.)
neapolitanum Schroder★
perpusillum Cleve★
polygonum Schütt★
pseudocrinitum Ostenf.★
pseudocurvisetum Mangin★
radians Schütt★
scolopendra Cleve★
seriacanthus Gran★
septentrionale Oestrup★
simile Cleve★
simplex Ostenf.★
skeleton Cleve★
sociale Laud.★
subsecundum (Grun.) Hust.★
(*distans* var. *subsecunda* Grun.)
(*paradoxus* Schütt)
(*paradoxus* var. *ludersii* Engler)
(*paradoxus* var. *subsecunda* Van Heurck)
(*ralfsii* Schütt)
(*groenlandicus* Cleve)
(*diadema* Gran)
(*Syndendrium diadema* Ehrenb.)
subtile Cleve★
teres Cleve★
tetrastichon Cleve★
tortissimum Gran★
wighami Brightw.★

Bacteriastraceae

BACTERIASTRUM Shadbolt, 1854.
delicatulum Cleve★
elongatum Cleve★
hyalinum Laud.★
solitarium Mangin★
varians Lauder★

Notes on Biddulphiineae

(1) This family is very ill defined and, despite the lengthy monograph by Boyer (1927), contains a wide range of forms whose only connexion is that they fail to fit reasonably into any other group of diatoms. In outline the cells may be circular, biangular or polygonal, and the valve surface is usually furnished with spines or processes. In such a family the synonymy is necessarily chaotic. The treatment adopted here is that of most modern taxonomists, and set out in Hendey (1937).

(2) Van Heurck (1896) and Mann (1907) include *Odontella* Agardh, *Lampriscus* Bail., *Zygoceros* Ehrenb., *Denticella* Ehrenb., *Pseudo-stictodiscus* Grun., *Amphitetras* Ehrenb., *Amphipentas* Ehrenb., *Cerataulus* Ehrenb., and *Triceratium* Ehrenb. The last two are recognized here (in part) as separate genera.

(3) Usually considered as a plankton species but frequently found in dense masses attached to a substratum.

(4) This species made its first appearance in the North Sea sometime between 1903 and 1907, and by 1909 had spread to the English Channel and the Irish Sea. It is now firmly established in British coastal waters.

(5) Not *Biddulphia spinosa* Grev.

(6) Placed here tentatively. The valve is without horn-like processes, and is not a true member of *Biddulphia* Gray. Mann (1907) placed it in *Trigonium* Cleve, but that is giving *Trigonium* a meaning not intended by its author; neither is it a *Triceratium* as I interpret that genus. This species is different from *T. striolatum* Roper.

(7) Included in *Biddulphia* by Van Heurck, but Hustedt separates it on account of the torsion of that cell in girdle view.

(8) *Eucampia groenlandica* Cleve is considered to be distinct from *E. zoodiacus* by Lebour (1930) and Boyer (1927). I follow Hustedt here in considering them synonymous. It has been suggested that they are seasonal variations of the same organism.

(9) Cleve (1873) created a new genus for this species on account of the absence of costae, a distinction not generally accepted.

(10) Hendey (1937) interprets the genus *Triceratium* Ehrenb. strictly in terms of *T. favus* the type of the genus, and excludes all species that do not possess cornutate processes and hexagonal areolation. This treatment reduces a very unwieldy genus to less than thirty species.

(11) *Trigonium* Cleve is reserved for species that have an areolate surface but whose angles are furnished with rounded bosses of fine pores.

(12) *Chaetoceros* is here considered as a neuter noun, and all specific epithets have been treated accordingly. All members of the genus are planktonic.

Suborder 5. SOLENIINEAE

Corethronaceae
CORETHRON Castracane, 1886. (1)
criophilum Castr.*
(*hystrix* Hensen)

Leptocylindraceae
BACTERIOSIRA Gran, 1900. (2)
fragilis Gran*
DACTYLIOSOLEN Castracane, 1886.
antarcticus Castr.*
mediterraneus Perag.*
DETONULA Schütt, 1893. (2)
confervacea (Cleve) Gran*
(*cystifera* Gran)
(*Lauderia confervacea* Cleve)
LAUDERIA Cleve, 1837. (2)
borealis Gran*
(*annulata* Cleve)
LEPTOCYLINDRUS Cleve, 1889.
danicus Cleve*
minimus Gran*
(*belgicus* Meun.)
SCHROEDERELLA Pavillard, 1913. (2)
delicatula (Perag.) Pav.*
(*Detonula delicatula* Gran)
(*Lauderia delicatula* Perag.)
schroderi (Berg.) Pav.* (3)

Rhizosoleniaceae
GUINARDIA Peragallo, 1892.
flaccida (Castr.) Perag.*
(*baltica* Schütt)
(*Rhizosolenia ? flaccida* Castr.)
(*R. castracanei* Cleve)
(*Henseniella baltica* Schütt)
RHIZOSOLENIA Brightwell, 1858. (4)
acuminata (Perag.) Gran
alata Brightw.*
var. *alata*
var. *indica* Ostenf.*
var. *inermis* (Castr.) Hust.*
bergonii Perag.*
(*amputata* Ostenf.)
calcar-avis Schultze*
castracanei Perag.*
cylindrus Cleve*
delicatula Cleve*
fragilissima Berg.*
(*faeroeensis* Ostenf.)
hebetata Bail.*
imbricata Brightw.*
(*striata* Grev.)
robusta Norm. ex Pritch.*
(*sigma* Schütt)
setigera Brightw.*
(*japonica* Castr.)
(*hensenii* Schütt)
shrubsolei Cleve*
stolterfothii Perag.*
(*Eucampia striata* Stolt.)
(*Pyxilla stephanos* Hensen)
styliformis Brightw.*

Notes on Soleniineae

(1) Hendey (1937) was able to show that all the published species of this genus should be considered as phases of one species. *Corethron hystrix* Hensen is therefore given as a synonym of *C. criophilum* Castr. The variation of this organism depends upon environmental factors of which probably salinity is the chief.

(2) Hendey (1937) placed this genus in Discineae; its transference to its present position is on account of its peculiar girdle formation, and follows Hustedt (1927–30) and Cleve-Euler (1951).

(3) Hustedt (1927–30) unites with *Schroderella delicatula* (Perag.) Pav. More information is required on British material.

(4) The genus *Rhizosolenia* Brightwell was accepted for conservation against *Rhizosolenia* Ehrenb., 1843, and *Monoceros* van Goor, 1824, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 72).

Suborder 6. ARAPHIDINEAE

Fragilariaceae
ASTERIONELLA Hassall ex W. Smith, 1856. (1)
bleakeleyii W. Sm.
formosa Hass.* (2)
japonica Cleve & Müller ex Gran* (3)
kariana Grun. in Cleve & Grun.* (4)
notata Grun. ex Van Heurck*
CAMPYLOSIRA Grunow, 1882.
cymbelliformis (Schm.) Grun. ex Van Heurck
(*Synedra cymbelliformis* Schm.)
CYMATOSIRA Grunow, 1862.
belgica Grun.
DIMEREGRAMMA Ralfs ex Pritchard, 1861.
fulvum (Greg.) Ralfs ex Pritch.
marinum (Greg.) Ralfs ex Pritch.
var. *marinum*
var. *lanceolatum* (Perag.) Hust.
minor (Greg.) Ralfs ex Pritch.
var. *minor*
var. *nana* (Greg.) Van Heurck
FRAGILARIA Lyngbye, 1819.
bicapitata Mayer

- FRAGILARIA (*cont.*)
  - *capucina* Desmaz. (2)
  - *construens* var. *pusilla* Grun.
    - var. *venter* (Ehrenb.) Grun.
  - *cylindrus* Grun.
  - *hyalina* (Kütz.) Grun.
  - *islandica* Grun. ex Van Heurck
  - *leptostauron* (Ehrenb.) Hust.
  - *oceanica* Cleve
  - *pinnata* Ehrenb.
    - var. *pinnata*
    - var. *trigona* (Brun. & Hérib.) Hust.
  - *striatula* Lyngb. (3)
  - *virescens* var. *oblongella* Grun.
- GLYPHODESMIS Greville, 1862.
  - *distans* (Greg.) Grun.
- OPEPHORA Petit, 1888.
  - *marina* (Greg.) Petit
    - (*Meridion marinum* Greg.)
  - *martyi* Hérib.
  - *pacifica* (Grun.) Petit
    - (*Fragilaria pacifica* Grun.)
  - *parva* (Grun.) Krasske
- PLAGIOGRAMMA Greville, 1859.
  - *brockmanni* Hust.
  - *gregoryanum* Grev.
  - *interruptum* (Greg.) Ralfs ex Pritch.
  - *laeve* (Greg.) Ralfs ex Pritch.
  - *staurophorum* (Greg.) Heib.
  - *vanheurckii* Grun.
- RHAPHONEIS Ehrenberg, 1844.
  - *amphiceros* Ehrenb. (5)
    - (*rhombus* Ehrenb.)
    - (*lanceolata* Ehrenb.)
    - (*amphiceros* var. *rhombica* Grun.)
  - *belgica* Grun.
    - var. *belgica*
    - var. *intermedia* Grun.
  - *minutissima* Hust.
  - *nitida* (Greg.) Grun.
  - *surirella* (Ehrenb.) Grun.
    - (*Zygoceros surirella* Ehrenb.)
- SCEPTRONEIS Ehrenberg, 1844. (6)
  - *caduceus* Ehrenb.
- TRACHYSPHENIA Petit, 1857.
  - *australis* Petit

Tabellariaceae

- GRAMMATOPHORA Ehrenberg, 1840.
  - *angulosa* Ehrenb.
  - *marina* (Lyng.) Kütz.
  - *oceanica* Ehrenb. (7)
    - var. *oceanica*
    - var. *macilenta* (W. Sm.) Grun.
    - var. *subtilissima* (Bail.) De Toni
  - *serpentina* Ehrenb.
- LICMOPHORA Agardh, 1827.
  - *anglica* (Kütz.) Grun.
  - *communis* (Heib.) Grun.
  - *dalmatica* (Kütz.) Grun.
  - *debilis* (Kütz.) Grun. ex Van Heurck
  - *ehrenbergii* (Kütz.) Grun.
  - *flabellata* (Carm.) Agardh (7)
  - *gracilis* (Ehrenb.) Grun. (7)
    - var. *gracilis*
    - var. *elongata* (Kütz.) de Toni
  - *hyalina* (Kütz.) Grun.
  - *juergensii* Agardh
  - *lyngbyei* (Kütz.) Grun. ex Van Heurck (7)
  - *ovata* (W. Sm.) Grun.
  - *paradoxa* (Lyngb.) Agardh
- RHABDONEMA Kützing, 1844.
  - *adriaticum* Kütz. (7)
  - *arcuatum* (Lyngb.) Kütz. (7)
  - *minutum* Kütz.
- STRIATELLA Agardh, 1832.
  - *delicatula* (Kütz.) Grun. ex Van Heurck
  - *interrupta* (Ehrenb.) Heib.
    - (*Tessella interrupta* Ehrenb.)
  - *unipunctata* (Lyngb.) Agardh
- SYNEDRA Ehrenberg, 1830.
  - *affinis* Kütz.
    - var. *affinis*
    - var. *fasciculata* Van Heurck
    - var. *gracilis* Grun.
    - var. *hybrida* Van Heurck
    - var. *parva* Kütz.
  - *barbatula* Kütz.
  - *crystallina* (Agardh) Kütz.
  - *formosa* Hantzsch
  - *fulgens* (Grev.) W. Sm. (7)
  - *gaillonii* (Bory) Ehrenb.
    - var. *gaillonii*
    - var. *macilenta* (Grun.) Perag.
  - *gracilis* Kütz.
  - *hennedeyana* Greg.* (8)
  - *hyperborea* Grun.
  - *investiens* W. Sm.
  - *pulchella* Kütz.
  - *robusta* Ralfs ex Pritch.
    - (*superba* Kütz.)
  - *tabulata* (Agardh) Kütz. (9)
    - (*fasciculata* Kütz.)
    - (*affinis* var. *tabulata* (Kütz.) Grun.)
    - var. *tabulata*
    - var. *acuminata* (Kütz.) Grun.
  - *ulna* (Nitzsch) Ehrenb.
  - *undulata* Bail.
- THALASSIONEMA Grunow ex Hustedt, 1932.
  - *nitzschioides* Hust.*
    - (*Thalassiothrix nitzschioides* Grun.)
- THALASSIOTHRIX Cleve & Grunow, 1880.
  - *frauenfeldii* Grun. in Cleve & Grun.*
  - *longissima* Cleve & Grun.*

Notes on Araphidineae

(1) Hassall (1850) first used the name *Asterionella* for what he described as a 'stelliform *Diatoma*'. A figure labelled *Asterionella formosa* was given but, as no generic description was provided, Hassall's name must be regarded as illegitimate. The authority for the genus is therefore given to Wm. Smith.

(2) Usually considered as a freshwater species, but frequently found in estuarine conditions.
(3) This species enjoys almost world-wide distribution, but usually favours warmer waters than those around our coasts.
(4) An arctic form. Further details are required about the southern limits of its distribution.
(5) The outline of this species is very variable.
(6) A doubtful record.
(7) Commonly epiphytic upon red algae, e.g. *Polysiphonia* and *Ceramium* spp.
(8) Usually favours warm water and a high salinity.
(9) Given by some authorities as a synonym of *Synedra affinis* Kütz. A more detailed examination of British material is required.

Suborder 7. RAPHIDIOIDINAE

Not represented.

Suborder 8. MONORAPHIDINEAE

Achnanthaceae

- ACHNANTHES Bory, 1822.
  - *affinis* Grun. in Cleve & Grun.
  - *angustata* Grev. (1)
  - *brevipes* Agardh (2)
    - var. *brevipes*
    - var. *intermedia* Kütz.
    - var. *parvula* (Kütz.) Cleve
      - (*A. parvula* Kütz.)
  - *clevei* var. *rostrata* Hust.
  - *coarctata* var. *sinoensis* Hust.
  - *delicatula* (Kütz.) Grun. in Cleve & Grun.
  - *hauckiana* Grun. (2)
    - var. *hauckiana*
    - var. *rostrata* Schulz
  - *lanceolata* (Bréb.) Grun.
    - var. *lanceolata*
      - f. *ventricosa* Hust.
    - var. *elliptica* Cleve
    - var. *rostrata* (Ostenf.) Hust.
  - *latissima* Cleve
  - *lemmermanni* Hust.
    - var. *lemmermanni*
    - var. *lineata* Salah
    - var. *obtusa* Hust.
  - *lilljeborgei* Grun.
  - *linkei* Hust.
  - *longipes* Agardh
  - *microcephala* (Kütz.) Cleve (3)
  - *pseudobrevipes* Aleem
  - *saxonica* Krasske
  - *similis* McCall
  - *subsessilis* Kütz. (2)
  - *taeniata* Grun. in Cleve & Grun.
- CAMPYLONEIS Grunow, 1862. (4)
  - *grevillei* (W. Sm.) Grun.
    - (*Cocconeis grevillei* W. Sm.)
- COCCONEIS Ehrenberg, 1838.
  - *clandestina* Schm.
  - *costata* Greg.
  - *diaphana* W. Sm.
  - *dirupta* Greg.
  - *distans* Greg.
  - *excentrica* Donk.
  - *fluminensis* (Grun.) Perag.
  - *grata* Schm.
  - *guttata* Hust. & Aleem
  - *molesta* var. *crucifera* Grun. ex Van Heurck
  - *norvegica* Grun.
  - *notata* Petit
  - *pelta* Schm.
  - *peltoides* Hust.
  - *placentula* Ehrenb.
  - *pseudomarginata* Greg.
  - *scutellum* Ehrenb.
    - var. *scutellum*
    - var. *ampliata* Grun.
    - var. *distans* (Greg.) Schm.
    - var. *ornata* Grun.
    - var. *parva* Grun. in Cleve
    - var. *stauroneiformis* W. Sm. in Cleve
  - *sublittoralis* Hendey (5)
  - *tenuis* Hust.
- RHOICOSPHENIA Grunow, 1860. (6)
  - *curvata* (Kütz.) Grun.
  - *marina* (W. Sm.) Schm.
  - *pullus* Schm. M.

Notes on Monoraphidineae

(1) Considered by some authorities as a variety of *Achnanthes brevipes* Agardh.
(2) This species and its varieties is seldom found in waters of full salinity, and in common with most brackish diatoms is liable to much variation in outline and size.
(3) Usually considered as a fresh-water species, but has been found in marine waters having a salinity of 33‰.
(4) Considered by some authorities to belong to *Cocconeis* Ehrenberg, but separated from that genus on account of a peculiar internal plate which is attached to the raphe-bearing valve.
(5) More information concerning the distribution of this species is required.
(6) Species of this genus were originally described as geniculate forms of *Gomphonema*. Further research, however, showed that the similarity with *Gomphonema* was superficial, and that the genus is correctly placed in the Monoraphidineae.

Suborder 9. BIRAPHIDINEAE

- **Naviculaceae** (1)
  - AMPHIPLEURA Kützing, 1844. (2)
    - *micans* Lyngb.
      - var. *micans*
      - var. *fragilis* (Grev.) Grun.
    - *rutilans* (Trent.) Cleve
      - (*Schizonema rutilans* Trent.)
  - AMPHIPRORA Ehrenberg, 1843.
    - *alata* (Ehrenb.) Kütz.
      - (*Navicula alata* Ehrenb.)
      - (*Amphicampa alata* Rab.)
    - *constricta* Ehrenb.
    - *didyma* W. Sm.
    - *gigantea* var. *sulcata* (O'Meara) Cleve
    - *hyalina* Eulen. ex Van Heurck
      - (*paludosa* var. *hyalina* Eulen. ex Van Heurck)
    - *hyperborea* Grun. in Cleve & Grun.*
    - *lata* Grev.
      - var. *lata*
      - var. *angustior* McCall
    - *ornata* Bail.
    - *paludosa* W. Sm.
      - var. *paludosa*
      - var. *duplex* Donk.
    - *pulchra* var. *pulchella* Perag.
    - *robusta* McCall
    - *sulcata* Perag.
    - *surirelloides* Hendey
    - *vitrea* W. Sm.
  - ANOMOEONEIS Pfitzer, 1871. (3)
    - *exellii* Salah
    - *sculpta* (Ehrenb.) Cleve
      - (*Navicula sculpta* Ehrenb.)
      - (*N. tumens* W. Sm.)
      - (*N. rostrata* Kütz.)
  - AURICULA Castracane, 1873. (4)
    - *complexa* (Greg.) Cleve
      - (*Amphiprora complexa* Greg.)
    - *decipiens* Grun.
    - *dubia* Perag.
    - *ostrea* Brun & Temp. (5)
  - BREBISSONIA Grunow, 1860. (2), (6)
    - *boecki* W. Sm.
  - CALONEIS Cleve, 1894. (3)
    - *amphisboena* (Bory) Cleve
      - (*Navicula amphisboena* Bory)
      - var. *amphisboena*
      - var. *fenzlii* (Grun.) Cleve
      - var. *liburnica* (Grun.) Cleve
      - var. *subsalina* (Donk.) Cleve
        - (*N. subsalina* Donk.)
    - *bicuneata* (Grun.) Boyer
      - (*N. bicuneata* Grun.)
    - *brevis* (Greg.) Cleve
      - (*N. brevis* Greg.)
    - *formosa* (Greg.) Cleve
      - (*N. formosa* Greg.)
      - var. *formosa*
      - var. *holmiensis* Cleve
    - *fusioides* (Grun.) Heid. & Kolbe
      - (*N. fusioides* Grun.)
      - (*N. inornata* Grun.)
      - (*N. subula* Grun.)
    - *hustedtii* Aleem in Aleem & Hust.
    - *lepidula* (Grun.) Cleve
      - (*N. lepidula* Grun.)
    - *liber* (W. Sm.) Cleve
      - (*N. liber* W. Sm.)
      - var. *liber*
      - var. *linearis* (Grun.) Cleve
      - var. *umbilicata* (Grun.) Cleve
  - DIPLONEIS Ehrenberg, 1840. (3), (7)
    - *advena* (Schm.) Cleve
      - var. *advena*
      - var. *sansegana* (Grun.) Cleve
    - *aestiva* (Donk.) Cleve
      - (*N. aestiva* Donk.)
    - *aestuari* Hust.
    - *bomboides* (Schm.) Cleve
      - (*N. bomboides* Schm.)
      - (*N. williamsonii* Van Heurck)
    - *bombus* Ehrenb.
      - (*N. bombus* Greg.)
      - (*N. abnormis* Castr.)
      - var. *bombus*
      - var. *egena* (Schm.) Cleve
    - *chersonensis* Grun.
      - (*N. apis* Ehrenb.)
    - *coffaeiformis* (Schm.) Cleve
    - *constricta* (Grun.) Cleve
      - (*N. constricta* Grun.)
      - (*N. donkinii* Schm.)
    - *contigua* var. *eudoxia* (Schm.) Cleve
      - (*N. mediterranea* Schm.)
      - (*N. eudoxia* Schm.)
      - var. *eugenia* (Schm.) Cleve
        - (*D. sejuncta* Jörg.)
        - (*N. eugenia* Schm.)
    - *crabro* Ehrenb.
      - (*N. crabro* Kütz.)
      - var. *crabro*
      - var. *expleta* (Schm.) Cleve
      - var. *pandura* (de Bréb.) Cleve
        - (*N. pandura* de Bréb.)
    - *cynthia* (Schm.) Cleve
      - (*N. cynthia* Schm.)
    - *didyma* (Ehrenb.) Cleve
      - (*Pinnularia didyma* Ehrenb.)
    - *divergens* (Schm.) Cleve
      - (*N. divergens* Schm.)
      - (*N. pfitzeriana* O'Meara)
    - *elliptica* (Kütz.) Cleve
      - (*N. elliptica* Kütz.)
    - *entomon* (Ehrenb.) Cleve
    - *fusca* (Greg.) Cleve
      - var. *fusca*
      - var. *delicata* Schm.
      - var. *gregorii* Cleve
      - var. *norvegica* Cleve
    - *hyalina* (Donk.) Cleve
      - (*N. hyalina* Donk.)
    - *incurvata* (Greg.) Cleve
      - (*N. incurvata* Greg.)

- Diploneis (*cont.*)
  - *incurvata* (*cont.*)
    - f. *incurvata*
    - f. *stricta* Hust.
  - *interrupta* (Kütz.) Cleve
    - (*N. interrupta* Kütz.)
  - *lineata* (Donk.) Cleve
    - (*N. lineata* Donk.)
    - (*N. adriatica* Grun.)
  - *littoralis* (Donk.) Cleve
    - (*N. littoralis* Donk.)
    - (*N. ovulum* Grun.)
  - *marginestriata* Hust.
  - *nitescens* (Greg.) Cleve
    - (*N. nitescens* Greg.)
    - var. *nitescens*
    - var. *candida* McCall
  - *notabilis* (Grev.) Cleve
  - *oamaruensis* (Cleve) Mills
    - (*adonis* var. *oamaruensis* Cleve)
    - (*N. oamaruensis* (Cleve) Mann)
  - *papula* (Schm.) Cleve
    - (*N. papula* Schm.)
    - var. *papula*
    - var. *constricta* Hust.
  - *parca* (Schm.) Boyer
  - *pseudovalis* Hust.
  - *puella* (Schum.) Cleve
  - *smithii* (de Bréb.) Cleve
    - (*N. smithii* de Bréb. in W. Sm.)
  - *splendida* (Greg.) Cleve
    - (*N. splendida* Greg.)
    - (*N. entomon* Donk.)
  - *stroemi* Hust.
  - *subcincta* (Schm.) Cleve
    - (*N. subcincta* Schm.)
  - *suborbicularis* (Greg.) Cleve
    - (*N. smithii* var. *suborbicularis* Greg.)
    - var. *suborbicularis*
    - var. *constricta* Hust.
  - *subovalis* Cleve
  - *vacillans* (Schm.) Cleve
    - var. *vacillans*
    - var. *delicatula* Cleve
    - var. *minuta* Grun.
  - *weissflogii* (Schm.) Cleve
- Donkinia Ralfs, 1860. (8)
  - *angusta* (Donk.) Ralfs ex Pritch.
    - (*Pleurosigma angustum* Donk.)
  - *carinata* (Donk.) Ralfs ex Pritch.
    - (*P. carinatum* Donk.)
  - *recta* (Donk.) Grun. ex Van Heurck
- Frustulia Agardh, 1824.
  - *rhomboides* var. *amphipleuroides* (Grun.) De Toni (9)
- Mastogloia Thwaites in W. Smith, 1856.
  - *apiculata* W. Sm. (10)
  - *binotata* (Grun.) Cleve
    - (*Cocconeis binotata* Grun.)
  - *danseii* (Thwaites) W. Sm.
  - *elliptica* (Agardh) Cleve
    - (*danseii* var. *elliptica* Van Heurck)
    - (*Frustulia elliptica* Agardh)
  - *exigua* Lewis
  - *lanceolata* Thwaites in W. Sm.
  - *ovata* Grun.
  - *ovulum* Hust.
  - *portierana* Grun.
  - *pumila* (Grun.) Cleve
  - *smithii* Thwaites in W. Sm.
    - var. *smithii*
    - var. *lacustris* Grun.
- Navicula Bory, 1822.
  - *abrupta* (Greg.) Cleve (11)
    - (*lyra* var. *abrupta* Greg.)
    - var. *abrupta*
    - var. *rattrayi* Pant.
  - *abstrusa* Hust.
  - *aequorea* Hust.
  - *aleemi* Hust.
  - *algida* Grun.
  - *ammophila* var. *flanatica* Grun.
  - *approximata* var. *niceaensis* (12)
    - (*hennedyii* var. *niceaensis* Perag.)
  - *arenicola* Grun.
  - *avenacea* de Bréb. (13)
    - (*viridula* var. *avenacea* (de Bréb.) Van Heurck)
  - *bacillum* Ehrenb.
  - *bahusiensis* Grun.
    - var. *bahusiensis*
    - var. *istriana* Grun. ex Van Heurck
  - *biskanteri* Hust.
  - *bottnica* Grun.
  - *bremeyeri* Hust.
    - var. *bremeyeri*
    - var. *rostrata* Hust.
  - *britannica* Aleem
  - *bulnheimii* var. *belgica* Grun.
  - *cancellata* Donk.
    - var. *cancellata*
    - var. *gregorii* Ralfs
    - var. *retusa* (de Bréb.) Cleve
      - (*N. retusa* de Bréb.)
    - var. *subretusa* (Ehrenb.) Van Heurck
  - *carinifera* Grun.
  - *cincta* Ehrenb.
    - var. *cincta*
    - var. *heufleri* Grun.
    - var. *leptocephala* (de Bréb.) Grun. ex Van Heurck
    - (*N. leptocephala* de Bréb.)
  - *clamans* Hust.
  - *clavata* Greg.
    - var. *clavata*
    - var. *indica* (Grev.) Cleve
    - var. *wrightii* (O'Meara) Perag. (14)
  - *claviculus* Greg.
  - *clementis* Grun.
  - *cluthensis* Greg. (15)
  - *complanata* Grun. in Cleve & Grun.
    - var. *complanata*
    - var. *subinflata* Grun.
  - *consentanea* Hust.
  - *corymbosa* (Agardh) Cleve
    - (*Schizonema corymbosum* Agardh)
  - *crucicula* (W. Sm.) Donk.
    - (*Stauroneis crucicula* W. Sm.)

- NAVICULA (*cont.*)
  - *crucicula* (*cont.*)
    - var. *crucicula*
    - var. *obtusata* Grun.
  - *crucifera* Grun.
    - (*N. rostellata* Schm.)
  - *crucigera* (W. Sm.) Cleve
    - (*Schizonema cruciger* W. Sm.)
    - (*Stauroneis crucigera* (W. Sm.) Heib.)
  - *cryptocephala* Kütz.
    - var. *cryptocephala*
    - var. *veneta* (Kütz.) Cleve
  - *cryptostriata* Salah
  - *cuspidata* Kütz.
  - *cuspis* O'Meara (16)
  - *cyprinus* (W. Sm.) Boyer (17)
    - (*digito-radiata* (Greg.) Ralfs)
    - (*digito-radiata* var. *cyprinus* (Ehrenb.) Van Heurck)
    - (*Pinnularia digito-radiata* Greg.)
    - var. *cyprinus*
    - var. *linearis* (Hust.)
    - var. *rostrata* (Hust.)
      - (*N. digito-radiata* var. *rostrata* Hust.)
  - *debilissima* Grun.
  - *detersa* Grun.
  - *dilucida* Hust.
  - *diploneoides* Hust.
  - *directa* (W. Sm.) Cleve (18)
    - (*Pinnularia directa* W. Sm.)
    - var. *directa*
    - var. *subtilis* Cleve
      - (*N. acutiuscula* Greg.)
      - (*Pinnularia subtilis* Greg.)
  - *diserta* Hust.
  - *distans* (W. Sm.) Cleve (19)
    - (*Pinnularia distans* W. Sm.)
  - *dunstonii* Salah
  - *elegans* W. Sm.
    - var. *elegans*
    - var. *cuspidata* Cleve (20)
  - *favus* Salah
  - *flagellifera* Hust.
  - *flanatica* Grun.
  - *forcipata* Grev.
    - var. *forcipata*
    - var. *densistriata* Schm. (21)
    - var. *punctata* Cleve
  - *formenterae* Cleve
  - *fortis* (Greg.) Ralfs ex Pritch.
    - (*Pinnularia fortis* Greg.)
    - (*P. constricta* O'Meara)
  - *fraudulenta* Schm.
  - *fuchsii* Pant. (22)
  - *gastrum* Ehrenb.
    - var. *gastrum*
    - var. *exigua* Greg.
  - *gelida* Grun.
    - (*kariana* var. *curta* Cleve)
  - *gotlandica* Grun.
  - *gracilis* var. *schizonemoides* Van Heurck
  - *granii* Jörg.*
    - (*Stauropsis granii* (Jörg.) Meun.)
  - *granulata* Bail. (23)
  - *gregaria* Donk.
  - *gregorii* Ralfs ex Pritch.
  - *grevillei* (Agardh) Cleve (24)
    - (*Schizonema grevillei* Agardh)
    - var. *grevillei*
    - var. *comoides* Aleem
  - *groschopfi* Hust.
  - *halophila* (Grun.) Cleve
    - (*cuspidata* var. *halophila* Grun.)
  - *hamulifera* Grun.
  - *hennedyii* W. Sm. (25)
    - var. *hennedyii*
    - var. *manca* Schm.
    - var. *nebulosa* (Greg.) Cleve (26)
      - (*N. nebulosa* Greg.)
  - *humerosa* de Bréb. in W. Sm.
    - var. *humerosa*
    - var. *arabica* Perag.
    - var. *constricta* Cleve
    - var. *minor* Heid.
  - *hungarica* var. *linearis* Östrup
  - *hyalosira* Cleve
  - *incerta* Grun. ex Van Heurck
  - *inflexa* (Greg.) Ralfs ex Pritch.
    - (*Pinnularia inflexa* Greg.)
  - *integra* (Smith) Ralfs
    - (*P. integra* Smith)
  - *jamalinensis* Cleve
  - *kryophila* Cleve
  - *lanceolata* (Agardh) Kütz.
    - var. *lanceolata*
    - var. *arenaria* (Donk.) Cleve
      - (*N. arenaria* Donk.)
    - var. *phyllepta* (Kütz.) Cleve
      - (*N. phyllepta* Kütz.)
  - *latissima* Greg.
  - *libellus* Greg.
  - *litoris* Salah
  - *longa* (Greg.) Ralfs ex Pritch.
    - (*P. longa* Greg.)
  - *lucens* Hust. ex Salah
  - *lunatapicalis* Salah
  - *lyra* Ehrenb. (27)
    - var. *lyra*
    - var. *atlantica* Schm.
    - var. *constricta* Perag.
    - var. *elliptica* Schm.
    - var. *granulata* Perag.
    - var. *subcarinata* Grun.
  - *maculosa* Donk.
  - *margino-nodularis* Salah
  - *marina* Ralfs ex Pritch. (28)
    - (*punctulata* W. Sm.)
  - *mediterranea* Cleve & Brun
  - *membranacea* Cleve*
    - (*Stauropsis membranacea* (Cleve) Meun.)
  - *mollis* (W. Sm.) Cleve (24)
    - (*Schizonema molle* W. Sm.)
  - *monilifera* Cleve (29)
    - (*granulata* de Bréb.)
    - var. *monilifera*
    - var. *heterosticha* Cleve
  - *moniliformis* Cleve

Navicula (*cont.*)
mutica Kütz.
northumbrica Donk.
obsidialis Hust.
octavosignata Salah
ostrearia Turp. (30)
(*fusiformis* Grun.)
palpebralis de Bréb. in W. Sm. (31)
var. *palpebralis*
var. *angulosa* (Greg.) Cleve
var. *barclayana* (Greg.) Cleve
var. *minor* (Greg.) Grun.
var. *obtusa* Van Heurck
var. *protracta* Perag.
var. *robusta* Heid.
var. *semiplena* (Greg.) Cleve
f. *vahliana* (Grun.) Hust.
pavillardi Hust.
pelagica Cleve*
(*Stauropsis pelagica* (Cleve) Meun.)
pennata Schm.
peregrina (Ehrenb.) Kütz.
(*Pinnularia peregrina* Ehrenb.)
var. *peregrina*
var. *kefvingensis* (Ehrenb.) Cleve
var. *meniscus* (Schum.) Cleve
plicata Donk.
praetexta Ehrenb.
producta McCall
protracta Grun.
pseudo-bacillum Grun.
pusilla W. Sm.
var. *pusilla*
var. *lanceolata* Grun.
pygmaea Kütz.
ramosissima (Agardh) Cleve (24)
(*Schizonema ramosissimum* Agardh)
var. *ramosissima*
var. *amplia* Grun.
var. *mucosa* Aleem
retusa de Bréb.
rhombica Greg. (32)
rhyncocephala Kütz.
var. *rhyncocephala*
var. *amphiceros* (Kütz.) Cleve
(*N. amphiceros* Kütz.)
salinarum Grun.
salinicola Hust.
scandinavica Lagerst.
scopulorum de Bréb. ex Kütz.
(*Pinnularia johnsonii* W. Sm.)
var. *scopulorum*
var. *belgica* (Van Heurck) Cleve
var. *perlonga* Brun
septentrionalis (Östrup) Cleve*
(*Libellus septentrionalis* Östrup)
(*Stauropsis septentrionalis* Meun.)
solaris Greg.
spectabilis Greg.
spicula (Hick.) Cleve
(*Stauroneis spicula* Hick.)
spuria Cleve
subinflata Grun.
supralitoralis Aleem & Hust.
ulvacea (Berk.) Cleve
(*Dickieia ulvacea* Berk.)
unilaterarea Salah
vanhöffeni Gran*
(*Stauropsis vanhöffeni* (Gran) Meun.)
viridula Kütz.
var. *viridula*
var. *rostellata* Kütz.
(*N. rostellata* Kütz.)
weissflogi Schm.
zohdyi Salah
zostereti Grun.

Oestrupia Heiden, 1906. (33)
musca (Greg.) Hust.
(*Navicula musca* Greg.)
(*Caloneis musca* (Greg.) Cleve)

Pinnularia Ehrenberg, 1843. (3)
ambigua Cleve
claviculus (Greg.) Cleve
(*Navicula claviculus* Greg.)
cruciformis (Donk.) Cleve
(*N. cruciformis* Donk.)
ergadensis Greg.
(*Navicula blanda* Schm.)
(*Caloneis blanda* (Schm.) Cleve)
var. *ergadensis*
var. *minor* (Perag.)
(*N. blanda* var. *minor* Perag.)
fritschii Salah
var. *fritschii*
var. *lata* Salah
quadratarea (Schm.) Cleve
(*Navicula quadratarea* Schm.)
(*N. pinnularia* Cleve)
var. *quadratarea*
var. *subproducta* (Grun.) Cleve
(*N. pinnularia* var. *subproducta* Grun.)
rectagulata (Greg.) Cleve
var. *rectangulata*
var. *subundulata* Grun.
stauntonii (Grun.) Cleve
(*Allioneis stauntonii* Grun.)
trevelyana (Donk.) Rab.
(*Navicula trevelyana* Donk.)

Pleurosigma W. Smith, 1852. (34)
acuminatum var. *brebissonii* (Grun.) Mills
aestuarii (de Bréb. ex Kütz.) W. Sm.
(*Navicula aestuarii* de Bréb. ex Kütz.)
affine Grun.
(*normanii* Ralfs ex Pritch.)
var. *affine*
var. *fossile* Grun.
angulatum (Quekett) W. Sm. (35)
(*Navicula angulata* Quekett)
var. *angulatum*
var. *finmarchica* Cleve
var. *quadratum* (W. Sm.) Cleve
(*P. quadratum* W. Sm.)
var. *robustum* McCall
attenuatum (Kütz.) W. Sm.
(*Frustulia attenuata* Kütz.)
var. *attenuatum*

- Pleurosigma (*cont.*)
  - *attenuatum* (*cont.*)
    - var. *scalprum* (Gaill. & Turp.) Cleve
      - (*Navicula scalprum* Gaill. & Turp.)
  - *balticum* (Ehrenb.) W. Sm.
    - (*Navicula baltica* Ehrenb.)
    - (*Gyrosigma balticum* (Ehrenb.) Rab.)
  - *cuspidatum* Cleve
  - *decorum* W. Sm.
  - *delicatulum* W. Sm.
    - var. *delicatulum*
    - var. *gracile* McCall
  - *diminutum* Grun.
    - (*Gyrosigma diminutum* (Grun.) Cleve)
    - var. *diminutum*
    - var. *constrictum* Grun.
      - (*P. reversum* Greg.)
  - *distortum* W. Sm.
    - (*Gyrosigma distortum* (W. Sm.) Cleve)
    - var. *distortum*
    - var. *undulatum* McCall
  - *elongatum* W. Sm.
  - *eximium* (Thwaites) Cleve
    - (*Colletonema eximia* Thwaites)
    - (*Gyrosigma eximium* (Thwaites) Boyer)
  - *fasciola* (Ehrenb.) W. Sm.
    - (*Ceratoneis fasciola* Ehrenb.)
    - var. *fasciola*
    - var. *closterioides* (Grun.) Perag.
    - var. *prolongatum* (W. Sm.) Grun. ex Van Heurck
    - var. *sulcatum* Grun.
  - *formosum* W. Sm.
    - (*australicum* Witt)
    - (*tahitense* Witt)
  - *hippocampus* (Ehrenb.) W. Sm.
    - (*Navicula hippocampus* Ehrenb.)
    - (*Gyrosigma hippocampus* Hass.)
  - *intermedium* W. Sm.
    - var. *intermedium*
    - var. *nubecula* (W. Sm.) Grun. ex Van Heurck
      - (*P. nubecula* W. Sm.)
  - *lanceolatum* Donk.
    - (*transversale* Roper)
  - *litorale* W. Sm.
  - *longum* var. *subrigidum* (Grun.) Perag.
  - *macrum* W. Sm.
    - (*Gyrosigma macrum* (W. Sm.) Cleve)
  - *marinum* Donk.
  - *maroccanum* Cleve (36)
    - (*Rhoicosigma maroccanum* Perag.)
  - *naviculaceum* de Bréb.
    - (*japonicum* Castr.)
    - (*transversale* W. Sm.)
  - *normani* Ralfs (37)
  - *obliquum* Grun. in Cleve & Grun.
    - (*Gyrosigma obliquum* (Grun.) Boyer)
  - *obscurum* W. Sm.
    - (*macilentum* Perag.)
  - *parkerii* Har.
    - (*Gyrosigma parkerii* (Har.) Cleve)
  - *rectum* Donk.
  - *rigidum* W. Sm.
    - var. *rigidum*
    - var. *giganteum* (Grun.) Cleve
      - (*P. giganteum* Grun.)
  - *rhombeum* Grun. in Cleve & Grun.
  - *speciosum* W. Sm.
  - *spencerii* W. Sm.
    - (*Gyrosigma spencerii* (W. Sm.) Cleve)
    - var. *spencerii*
    - var. *exile* Grun.
    - var. *smithii* Grun. in Cleve & Grun.
  - *strigilis* W. Sm.
    - (*Gyrosigma strigilis* (W. Sm.) Cleve)
  - *strigosum* W. Sm. (38)
    - (*angulatum* var. *strigosum* Cleve)
  - *subhyalinum* Hust. & Aleem
  - *sulcatum* Grun.
  - *tenuissimum* W. Sm.
    - (*Gyrosigma tenuissimum* (W. Sm.) Cleve)
  - *wansbeckii* Donk.
    - (*balticum* var. *wansbeckii* (Donk.) Van Heurck)
- Scoliopleura Grunow, 1860.
  - *tumida* (de Bréb. ex Kütz.) Rab.
    - (*Navicula jennerii* W. Sm.)
    - (*N. tumida* (de Bréb.) Cleve)
    - var. *tumida*
    - var. *adriatica* (Grun.) Cleve
  - *westii* (W. Sm.) Grun.
    - (*N. westii* W. Sm.)
- Scoliotropis Cleve, 1894.
  - *latestriata* (de Bréb. ex Kütz.) Cleve (39)
    - (*Amphiprora latestriata* de Bréb. ex Kütz.)
    - (*Navicula convexa* W. Sm.)
- Stauroneis Ehrenberg, 1843. (40)
  - *acuta* W. Sm.
    - (*kochii* Pant.)
  - *africana* var. *acuminata* Grun.
  - *amphioxys* Greg. (41)
    - (*gregorii* Ralfs ex Pritch.)
  - *amphoroides* Grun.
  - *salina* W. Sm.
  - *septentrionalis* Grun.
- Stenoneis Cleve, 1894. (42)
  - *inconspicua* (Greg.) Cleve
    - (*Navicula inconspicua* Greg.)
    - (*N. fistula* Schm.)
- Toxonidea Donkin, 1858. (43)
  - *gregoryana* Donk.
  - *insignis* Donk.
    - var. *insignis*
    - var. *undulata* Norm.
- Trachyneis Cleve, 1894. (3)
  - *aspera* (Ehrenb.) Cleve
    - (*Navicula aspera* (Ehrenb.) Donk.)
    - (*Stauroptera aspera* Ehrenb.)
    - var. *aspera*
    - var. *intermedia* (Grun.) Cleve
    - var. *pulchella* (W. Sm.) Cleve
      - (*Stauroneis pulchella* W. Sm.)
      - (*S. pygmaea* Castr.)
  - *clepsydra* (Donk.) Cleve

- Trachyneis (*cont.*)
  - *clepsydra* (*cont.*)
    - (*Navicula clepsydra* Donk.)
    - var. *clepsydra*
    - var. *scotica* Schm.
- Tropidoneis Cleve, 1891.
  - *elegans* (W. Sm.) Cleve
    - (*Amphiprora elegans* W. Sm.)
  - *gibberula* Grun.
  - *lepidoptera* (Greg.) Cleve
    - (*A. lepidoptera* Greg.)
  - *maxima* (Greg.) Cleve
    - (*A. maxima* Greg.)
    - var. *maxima*
    - var. *dubia* (Cleve & Grun.) Cleve
      - (*A. maxima* var. *dubia* Cleve & Grun.)
  - *pusilla* (Greg.) Cleve
    - (*A. pusilla* Greg.)
  - *recta* (Greg.) Cleve
    - (*A. recta* Greg.)
  - *vanheurckii* (Grun.) Cleve
    - (*Plagiotropis vanheurckii* Grun.)
  - *vitrea* (W. Sm.) Cleve
    - (*Amphiprora vitrea* W. Sm.)
    - (*Plagiotropis vitrea* Grun.)

Cymbellaceae

- Amphora Ehrenberg, 1831.
  - *acuta* Greg.
  - *acutiscula* Kütz.
    - (*lineata* Greg.)
  - *angularis* Greg.
  - *angusta* Greg.
  - *arcus* Greg. (44)
  - *arenaria* Donk.
    - var. *arenaria*
    - var. *donkinii* Rab.
    - var. *rattrayi* Cleve
  - *arenicola* Grun.
    - var. *arenicola*
    - var. *minor* McCall
  - *bacillaris* Greg. (45)
  - *binodis* Greg.
  - *coffeaeformis* (Agardh) Kütz.
    - (*aponina* Kütz.)
    - (*Frustulia coffeaeformis* Agardh)
    - var. *coffeaeformis*
    - var. *borealis* (Kütz.) Cleve
      - (*A. borealis* Kütz.)
    - var. *perpusilla* (Grun.) Cleve
  - *costata* W. Sm. (46)
  - *crassa* Greg. (47)
  - *cymbifera* Greg. (48)
  - *decussata* Grun.
  - *dubia* Greg. (49)
  - *elliptica* (Agardh) Kütz.
  - *ergadensis* Greg. (50)
  - *eunotia* Cleve
    - (*cymbifera* Cleve)
  - *excisa* Greg. (51)
  - *exigua* Greg. (52)
  - *fluminensis* Grun. (53)
  - *graeffi* (Grun.) Cleve (54)
    - var. *graeffi*
    - var. *minor* Perag.
  - *granulata* Greg.
  - *grevilleana* Greg.
    - (*fasciata* Greg.)
    - (*complexa* Greg.)
    - (*sulcata* Greg.) ?
  - *hyalina* Kütz.
    - (*hemisphaerica* Grun.)
  - *laevis* Greg.
    - (*nobilis* Flögel)
  - *laevissima* Greg. (55)
  - *lineolata* Ehrenb. (56)
  - *lyrata* Greg. (57)
  - *macilenta* Greg.
  - *marina* W. Sm. (58)
  - *membranacea* W. Sm. (59)
  - *milesiana* Greg. (60)
  - *monilifera* Greg. (61)
  - *nobilis* Greg. (62)
  - *oblonga* Greg. (63)
  - *obtusa* Greg. (64)
    - var. *obtusa*
    - var. *rectangularis* Perag.
  - *ocellata* Donk.
    - var. *ocellata*
    - var. *singulata* Cleve
  - *ostrearia* de Bréb. (65)
    - (*littoralis* Donk.)
    - var. *ostrearia*
    - var. *lineata* Cleve
    - var. *vitrea* Cleve
  - *ovalis* Kütz.
    - (*affinis* Kütz.)
    - (*lybica* (Ehrenb.)
    - (*ovalis* var. *lybica* (Ehrenb.) Cleve)
    - (*Navicula amphora* Ehrenb.)
  - *proboscidea* Greg. (66)
  - *proteus* Greg.
    - (*hexagonalis* Witt) ?
    - (*speciosa* Castr.) ?
    - var. *proteus*
    - var. *oculata* Perag.
  - *pusilla* Greg. (67)
  - *pusio* Cleve
  - *quadrata* Greg. (68)
  - *rhombica* Kitt.
  - *robusta* Greg. (69)
  - *salina* W. Sm. (70)
  - *spectabilis* Greg.
  - *sulcata* de Bréb. (71)
  - *tenera* W. Sm. (72)
  - *tenerrima* Aleem & Hust.
  - *turgida* Greg. (53)
  - *valida* Perag.
  - *veneta* Kütz.
  - *ventricosa* Greg.
- Okedenia Eulenstein ex De Toni, 1891. (73)
  - *inflexa* (de Bréb.) De Toni
    - (*Amphipleura inflexa* de Bréb. ex Kütz.)
    - (*Amphora inflexa* (de Bréb.) Cleve)

Gomphonemaceae

- Gomphonema Hustedt in Pascher, 1930.
  - *exiguum* Kütz.

Epithemiaceae

- EPITHEMIA de Brébisson, 1844.
  - *adnata* var. *proboscidea* (Grun.)
    - (*zebra* var. *proboscidea* Kütz.)
  - *sorex* Kütz.
  - *turgida* var. *westermanii* (Ehrenb.) Grun.
- RHOPALODIA O. Müller, 1895.
  - *gibba* (Ehrenb.) Müll.
    - (*Navicula gibba* Ehrenb.)
    - (*Epithemia gibba* Kütz.)
    - var. *gibba*
    - var. *ventricosa* (Kütz.) Grun.
  - *gibberula* var. *producta* (Grun.) Müll.
  - *musculus* (Kütz.) Müll.
    - (*Epithemia musculus* Kütz.)
    - var. *musculus*
    - var. *constricta* (de Bréb.) Müll.

Bacillariaceae

- BACILLARIA Gmelin, 1778. (74)
  - *paxillifer* (Müll.) Hendey
    - (*paradoxa* Gmelin)
    - (*Nitzschia paxillifer* (Müll.) Heib.)
    - (*Vibrio paxillifer* Müll.)
  - *socialis* Ralfs
    - (*Nitzschia socialis* Greg.)
    - var. *socialis*
    - var. *baltica* Grun.
- CYLINDROTHECA Rabenhorst, 1859. (75)
  - *gracilis* (de Bréb.) Grun.
- HANTZSCHIA Grunow in Cleve & Grunow, 1880. (76)
  - *amphioxys* var. *minor* Perag.
  - *hyalina* Grun.
  - *marina* (Donk.) Grun. in Cleve & Grun.
  - *rigida* McCall
  - *virgata* (Roper) Grun.
    - var. *virgata*
    - var. *gracilis* Hust.
- NITZSCHIA Hassall, 1845. (77)
  - *acicularis* (Kütz.) W. Sm.
    - (*Synedra acicularis* Kütz.)
  - *acuminata* (W. Sm.) Cleve in Cleve & Grun.
    - (*Tryblionella acuminata* W. Sm.)
    - var. *acuminata*
    - var. *subconstricta* Grun.
  - *aequorea* Hust.
  - *affinis* Grun.
  - *amphibia* Grun.
  - *angularis* W. Sm.
  - *apiculata* (Greg.) Grun. in Cleve & Grun.
    - (*Tryblionella apiculata* Greg.)
  - *bilobata* W. Sm.
    - var. *bilobata*
    - var. *minor* Grun.
  - *calcicola* Aleem & Hust.
  - *calida* var. *salinarum* (Grun.) Freng.
    - (*N. tryblionella* var. *salinarum* Grun.)
  - *capitellata* Hust.
  - *circumsuta* (Bail.) Grun. in Cleve & Grun.
    - (*Surirella circumsuta* Bail.)
  - *clausii* Hantzsch
  - *closterium* (Ehrenb.) W. Sm.* (78)
    - (*Ceratoneis closterium* Ehrenb.)
  - *commutata* Grun.
  - *constricta* Ralfs ex Pritch.
    - var. *constricta*
    - var. *subconstricta* Grun.
  - *debilis* (Arnott) Grun.
    - (*Tryblionella debilis* Arnott)
  - *delicatissima* Cleve*
    - (*Pseudonitzschia delicatissima* (Cleve) Heiden)
  - *denticula* var. *delognei* Grun. ex Van Heurck
  - *distans* Greg.
  - *dubia* W. Sm.
  - *dubiformis* Hust.
  - *epithemioides* Grun. in Cleve & Grun.
  - *filiformis* (W. Sm.) Schütt
    - (*Homoeocladia filiformis* W. Sm.)
  - *fonticola* Grun.
  - *frigida* Grun. in Cleve & Grun.
  - *frustulum* (Kütz.) Grun. in Cleve & Grun.
    - (*Synedra frustulum* Kütz.)
  - *gotlandica* A. Cleve-Euler
  - *granulata* Grun. in Cleve & Grun.
  - *habirshawii* Feb.
  - *hungarica* Grun.
  - *hustedtiana* Salah
  - *hybrida* Grun.
  - *incurva* Grun. in Schneider
  - *insignis* Greg.
    - var. *insignis*
    - var. *smithii* (Ralfs) Pell.
      - (*N. spectabilis* W. Sm.)
  - *irregularis* Ross & Abdin
  - *laevis* Hust.
  - *lanceolata* W. Sm.
    - var. *lanceolata*
    - var. *incrustans* Grun.
  - *linkei* Hust.
  - *littoralis* Grun. in Cleve & Grun.
    - var. *littoralis*
    - var. *slesvicensis* Grun.
  - *longissima* (de Bréb.) Ralfs ex Pritch.
    - (*birostrata* W. Sm.)
    - (*Ceratoneis longissima* de Bréb.)
  - *lorenziana* Grun.
    - var. *lorenziana*
    - var. *subtilis* Grun.
  - *macilenta* Greg.
  - *marginulata* var. *didyma* Grun. in Cleve & Grun.
  - *marina* Grun. in Cleve & Grun.
  - *martiana* (Agardh) Schütt
    - (*Homoeocladia martiana* Agardh)
  - *microcephala* Grun. in Cleve & Grun.
  - *navicularis* Grun. in Cleve & Grun.
    - (*Surirella navicularis* de Bréb.)
    - (*Tryblionella marginata* W. Sm.)
  - *obtusa* W. Sm.
    - var. *obtusa*
    - var. *scalpelliformis* Grun. in Cleve & Grun.

NITZSCHIA (*cont.*)
- *panduriformis* Greg.
    - var. *panduriformis*
    - var. *minor* Grun.
- *paravasa* Grun.
- *parvula* W. Sm.
- *petitiana* Grun. ex Van Heurck
- *plana* W. Sm.
- *polaris* Grun.
- *punctata* (W. Sm.) Grun.
    - var. *punctata*
    - var. *coarctata* Grun.
- *pungens* Grun.
- *reversa* W. Sm. (78)
- *salinicola* Aleem & Hust.
- *seriata* Cleve*
    - (*Pseudonitzschia seriata* (Cleve) Perag.)
- *sigma* (Kütz.) W. Sm.
    - (*Synedra sigma* Kütz.)
    - var. *sigma*
    - var. *intercedens* Grun.
    - var. *rigida* Grun.
    - var. *rigidula* Grun.
    - var. *sigmatella* Grun.
- *sigmoidea* (Nitzsch) W. Sm.
    - (*Bacillaria sigmoidea* Nitzsch)
    - (*Navicula sigmoidea* Ehrenb.)
- *spathulata* de Bréb. ex W. Sm.
    - var. *spathulata*
    - var. *hyalina* (Greg.) Grun. in Van Heurck
- *spectabilis* (Ehrenb.) Ralfs
    - (*brebissonii* W. Sm.)
- *subcapitellata* Hust.
- *subfrustulum* Hust.
- *tenuissima* Perag.
- *thermalis* var. *littoralis* Grun.
    - var. *minor* Hilse
- *tryblionella* Hantz.
    - var. *tryblionella*
    - var. *levidensis* (W. Sm.) Grun.
    - var. *maxima* Grun.
    - var. *recta* McCall
- *valdestriata* Aleem & Hust.
- *vasta* Hust.
- *vivax* W. Sm.

Notes on Biraphidineae

(1) The Naviculaceae is the largest family of diatoms and the genera recognized here follow the review of the naviculoid diatoms by Cleve (1894–5). Cleve instituted several new genera and recognized older genera created by Ehrenberg in order to reduce a very large genus to a more manageable size. In the following year Van Heurck (1896) ignored Cleve's work, and absorbed Cleve's genera into the genus *Navicula* Bory, which he divided into twenty-two groups. De Toni (1891–4) and Mann (1907) to a large degree followed Van Heurck, but most modern taxonomists follow Cleve.

(2) Mann (1907) includes in *Frustulia* Agardh. Van Heurck and De Toni consider it a separate genus.

(3) Included in *Navicula* by Van Heurck, see (1) above. The generic name *Pinnularia* Ehrenb., 1843, was accepted for conservation against *Pinnularia* Lindl. & Hutt., 1833, and *Stauroptera* Ehrenb., *Ber. Akad. Wiss., Berl.*, 1843, p. 45, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 71).

(4) Seldom found in large quantities. A critical review of the genus is required to define specific differences and geographical ranges. The generic name *Auricula* Castracane, 1873, was accepted for conservation against *Auricula* Spach, 1840, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 70).

(5) Recently observed in Chichester Harbour (Hendey, 1951), but originally described from a fossil deposit in Japan.

(6) Appears to be more common along the north and west than other coasts of Great Britain. The name *Brebissonia* Grunow was accepted for conservation against *Brebissonia* Spach, 1835, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 70).

(7) It is accepted to-day by all diatomists that *Diploneis* Ehrenb. is sufficiently distinct to be recognized as a separate genus, despite the fact that Van Heurck and Mann included it in *Navicula* Ehrenb. However, a certain amount of confusion exists in the genus, largely because so many of the species were described in early literature and, by modern standards, poorly illustrated. Many of Ehrenberg's species are unidentifiable and the synonymy is complicated. Most of the species vary greatly in size and appearance, and this has led to an unwarranted multiplicity of names. The genus requires a critical review and the distribution of each species defined.

(8) The sigmoid diatoms are dealt with here after the manner of Peragallo in 'Monographie du Genre *Pleurosigma*' published in *Le Diatomiste* (1890–1). In *Donkinia* the raphe is keeled above the valve surface and usually strongly sigmoid, while the valve outline is seldom sigmoid or only weakly so.

(9) Usually recognized as a fresh-water species, but this variety is found not infrequently in fully saline waters.

(10) The genus *Mastogloia* is recognized by most diatomists to-day, although Mann (1907) grouped it under *Navicula*. It is distinguished from the latter by the presence of a loculiferous rim which is attached to the inside of the girdle. This internal system often becomes detached during cleaning operations and the valves without it differ in no way from those of *Navicula*. The genus is not well represented in British waters, and further research is needed on the distribution of the species.

(11) Common around the west coasts of Britain, and usually considered distinct from *N. lyra*.

(12) *N. approximata* Greville is sharply separated from other members of the Naviculae Lyratae by the foreshortening of the median striae around the raphe at the central nodule. This character is clearly shown in Peragallo's figure of *N. hennedyii* var. *niceaensis* (*Diat. Mar. France*, 1897–1908, pl. 24, fig. 19, 15).

(13) Connected by intermediate forms to *N. viridula* Kütz.

(14) Connected by intermediate forms to *N. lyra* Ehrenb.

(15) Cleve makes this a variety of *N. punctulata* W. Sm.

(16) Doubtful species.

(17) The shape of the valve apex and the gibbous sides of this organism are very variable characters, and much confusion in the synonymy has arisen by creating varietal names to accommodate the differences seen. Most of these are here included under the earliest valid name of *N. cyprinus* (*Pinnularia cyprinus* Ehrenb., 1842).

(18) A very variable species, the exact limits of which should be more accurately defined.

(19) This species is a cold-water form, and the southerly limits of its distribution require defining.

(20) A large gathering of *N. elegans* shows much variation of the valve apices, and var. *cuspidata* with rostrate apices is often linked to the type by a series of intermediate forms.

(21) Usually more finely striate than the type, but the var. is of doubtful value.

(22) A small form of this species (about half the size of specimens found on American coasts) was found at Anglesey. This is the first record for the British Isles. It is closely allied to *N. humerosa* de Bréb.

(23) Owing to Bailey's poor illustration, this species frequently has been confused with *N. brasiliensis* Grun. and other allied species. *N. granulata* Bail. has rounded apices and interrupted striation. It is probably a cold-water form.

(24) This species lives in large frondose colonies of mucous tubes in conditions of reduced salinity.

(25) A very variable species, under which has been gathered a host of forms in which the marginal striae vary greatly in width. Smith did not publish a figure, but in his type specimen the marginal striate portion is less wide than the lateral area. Specimens from British coasts usually conform very well to the type.

(26) It is possible that this should be regarded as a distinct species.

(27) The type specimen was described from washings of seaweeds from the Falkland Islands in 1843, and since that time over thirty varietal names have been published. Some authors refuse to accept these names and refer all varieties to the type. The species is very variable, and a competent monograph on it is badly needed. It is not numerous around British coasts and, although the varieties listed are fairly readily recognized, their inclusion here is tentative.

(28) First described by Wm. Smith in 1853 as *N. punctulata*, a name previously used by Ehrenberg in 1842 to describe another species.

(29) First described by de Brébisson as *N. granulata*, a name previously used by Bailey in 1854 to describe another species.

(30) This species is commonly found where oysters are cultured. The cytoplasm towards the apices of the cell frequently assumes a blue colour.

(31) A widely distributed species, showing many varieties of somewhat doubtful value. More information required on distribution and ecology.

(32) Should be compared with *N. grevillei* (Agardh) Cleve.

(33) The genus *Oestrupia* Heiden, based upon *Caloneis powelli* (Lewis) Cleve, is connected to *Pinnularia* Ehrenberg and *Caloneis* Cleve. Hustedt (1935) compares and contrasts the valve structure of these genera, and shows that the transverse costae of *Oestrupia* are interrupted by a longitudinal siliceous band upon either side of the raphe.

(34) The genus *Pleurosigma* W. Sm. here includes species whose valves have striae crossing at right angles as well as those crossing obliquely. Some authorities refer the former to *Gyrosigma* Hassall; no useful purpose is however served by this. The generic name *Pleurosigma* W. Sm. was accepted for conservation against *Scalprum* Corda, 1835, *Gyrosigma* Hassall, 1845, and *Endosigma* de Bréb. ex D'Orb., 1849, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 72).

(35) All varieties of this species are of doubtful value, as intermediate forms connect them, producing a continuous series.

(36) A doubtful species.

(37) Probably *P. affine* Grun.

(38) Electron micrographs of this species show that its ultimate structure is entirely different from that of *P. angulatum*, and that Cleve was in error in relating them.

(39) More information is required about the distribution of this species.

(40) *Stauroneis* Ehrenberg was included in *Navicula* Ehrenberg by Van Heurck and Mann but most modern taxonomists separate them.

(41) Gregory's epithet must replace the later one of Ralfs which has been normally used for this species.

(42) In *Stenoneis* Cleve the raphe is enclosed between two stout ribs. One species is represented in the British flora. More information is required concerning its distribution.

(43) Included by some authorities in *Pleurosigma* but separated here on account of its bow-shaped raphe.

(44) Several varieties of this species have been described but intermediate forms appear to connect them. The published figures are of poor quality and more information is required concerning the whole group.

(45) The description provided by Gregory is not precise and the species requires a more accurate examination.

(46) This may be identical with *A. monilifera* Greg.

(47) Several varieties of this species are known but intermediate forms connect them. More information is required.

(48) Cleve (1895) makes this a synonym of *A. terroris* Ehrenberg.

(49) Doubtful species.

(50) Cleve (1895) makes this species a variety of *A. macilenta*, but the identification from the figures provided is doubtful. This species is akin to *A. acutiuscula* but its striae are coarser.

(51) This may be the same as, or related to, *A. laevis*.

(52) Gregory's figure does not admit of trustworthy identification. This species is related to *A. acutiuscula*.

(53) According to Cleve (1895) *A. fluminensis* Grun. agrees with *A. turgida* Greg.

(54) This species is probably the same as *A. grevilleana* but the striae are closer and not distinctly punctate.

(55) Considered by some authorities to be a variety of *A. laevis*.

(56) There seems to be some doubt as to the identity of this species. The figures provided by Ehrenberg and Kützing are not very satisfactory. *A. lineolata* in Donkin represents another species.

(57) This may be identical with *A. cuneata* Cleve.

(58) A doubtful species, probably a form of *A. proteus*.

(59) Probably the same as *A. ostrearia* de Bréb.

(60) This species is closely allied to *A. exsecta* Grun.

(61) Probably identical with *A. costata*.

(62) This may be the same as *A. acuta*.

(63) Seems to be a form of *A. robusta*. More work is required on this.

(64) Very variable species. More work is required on the distribution of this species and its variety.

(65) The varieties at first sight appear very dissimilar, but intermediate forms connect them to the type to such a degree that some authorities think they should be united.

(66) Cleve (1895) considers this nearly akin to *A. graeffi*.

(67) A doubtful species, very similar to *A. bacillaris* Greg.

(68) Doubtful species, allied to *A. truncata* Greg.

(69) Related to *A. proteus* but differs from it principally by coarser striae and larger size.

(70) May be the same as *A. coffaeaformis*.

(71) A doubtful species, probably connected to *A. elongata* Greg.

(72) Cleve (1895) considers this may be a small form of *A. lineolata*. More information is required on this.

(73) The generic name was originally attached to specimens by Eulenstein, and did not receive valid publication until 1891 when it appeared in De Toni's *Sylloge Algarum*. Cleve included *Okedenia inflexa* in *Amphora* but the organism bears no relation to that genus. More information is required on its distribution.

(74) The genus *Bacillaria* has been accepted by most taxonomists because of the characteristic mode of colony formation adopted by the type species *B. paxillifer*. The cells join together by their valve faces to form packets and move by sliding one on the other

somewhat like a slide rule. As colony formation and methods of movement are no longer considered as valid characters on which to base genera, *Bacillaria* is retained here on account of the symmetry of the frustule. It is distinguished from the genus *Nitzschia* Hassall in that the raphe is central and in the apical axis of the valve, whereas in *Nitzschia* it is marginal and the two raphes are placed diagonally upon the frustule.

(75) Not commonly found in marine waters. A spindle-shaped nitzschioid diatom in which the frustule is twisted about the apical axis so that the raphes appear helical.

(76) The genus *Hantzschia* is separated from *Nitzschia* because the raphe-bearing keels occupy adjacent margins of the frustule (see note 74).

(77) *Nitzschia* Hassall (1845) is an absolute synonym of *Sigmatella* Kützing (1833), as both genera were based on *Bacillaria sigmoidea* Nitzsch. The genus *Nitzschia* is accepted here in the sense that most modern taxonomists have used it, that is, in the sense that Wm. Smith (1853) used it taking *N. sigmoidea* Wm. Smith based on *Sigmatella nitzschii* Kützing, which was *Bacillaria sigmoidea* Nitzsch, as the type of the genus. Grunow in Cleve & Grunow (1880) divided the genus into twenty-four groups, most of which are generally accepted. British material requires much more detailed examination and revision. The generic name *Nitzschia* was accepted for conservation at the 7th Int. bot. Congr., Stockholm (*Int. Code bot. nomencl.*, 1952, p. 71).

(78) Some authorities make this a variety of *Nitzschia longissima*. The group is a difficult one as the diatoms are not strongly siliceous. More information is required on the British material.

Suborder 10. SURIRELLINEAE

Surirellaceae (1)
  CAMPYLODISCUS Ehrenberg, 1841.
    bicostatus W. Sm.
    clypeus Ehrenb. (2)
      (*ovatus* Ralfs)
      (*Cocconeis clypeus* Ehrenb.)
      (*Surirella clypeus* Ehrenb.)
    decorus de Bréb.
    echineis Ehrenb. (2)
      (*argus* Bail.)
      (*cribrosus* W. Sm.)
      (*Coronia echineis* Ehrenb.)
    fastuosus Ehrenb.
      (*parvulus* W. Sm.)
      (*thuretii* de Bréb.)
    hodgsonii W. Sm.
      (*eximius* Greg.)
    hypodromus Brun & Temp.
      (*gregoryii* Perag.)
    innominatus Ross & Abdin
    ralfsii W. Sm.
  PODOCYSTIS Bailey ex Wm. Smith, 1856. (3)
    adriatica (Kütz.) Boyer
      (*americana* Bail.)
      (*Surirella adriatica* Kütz.)
      (*Euphyllodium spathalatum* Shad.)
  SURIRELLA Turpin 1828. (4)
    caspia Brun
      (*cardaria* Brockm.)
    constricta Ehrenb.
    crumena de Bréb. ex Kütz.
      (*brightwellii* W. Sm.)
    fastuosa Ehrenb.
      (*hohenackerii* Rab.)
      (*cuneata* Schm.)
      var. *fastuosa*
      var. *cuneata* Witt.
    gemma (Ehrenb.) Kütz.
    hispida Ross & Abdin
    lata W. Sm. (5)
      (*hybrida* Grun.)
    minima Ross & Abdin
    ovalis de Bréb.
    ovata Kütz.
      var. *ovata*
      var. *salina* (W. Sm.) Hust.
    pyriformis Kitt.
    striatula Turp. (6)
      (*Navicula striatula* (Turp.) Ehrenb.)
    strigosa Hust.
    subsalsa W. Sm.

Notes on Surirellineae

(1) Some authorities regard the genera described here as belonging to the Biraphidineae, but the type of raphe and its peripheral position on the valve separate them most definitely from those genera that possess a single raphe in the axial area of each valve.

(2) Not abundant in British waters, more information is required concerning distribution and ecology.

(3) This genus is sometimes ascribed to Kützing (Kützing, 1844, p. 62), but the name was not validly published there. The generic name *Podocystis* Bailey ex W. Smith was accepted for conservation against *Euphyllodium* Shadbolt, 1854, by the Special Committee for Diatomaceae (*Int. Code bot. nomencl.*, 1952, p. 72).

(4) The smaller species of this genus lack precise descriptions, and it is likely that a thorough review of the group would considerably reduce the number of names now in use.

(5) Some authorities consider this to be identical with *Surirella fastuosa*.

(6) This species is very variable and several worthless varieties have been made.

NEW COMBINATIONS

During the course of this work it became necessary to make a number of new combinations. In order to validate them they are set out below together with synonyms and references.

Actinocyclus octonarius var. *crassus* (W. Smith) Hendey, *comb.nov.*

Eupodiscus crassus W. Smith, *Syn. Brit. Diat.*, **1**, 24 (1853).
Actinocyclus crassus (W. Smith) Ralfs, in Pritchard, *Hist. Infus.*, ed. 4, 835 (1861).
Actinocyclus ehrenbergii var. *crassus* (W. Smith) Hustedt, in Rabenhorst, *Krypt.-Flora*, **7** (1), 529 (1929).

Actinocyclus octonarius var. *ralfsii* (W. Smith) Hendey, *comb.nov.*

Eupodiscus ralfsii W. Smith, *Syn. Brit. Diat.*, **2**, 86 (1856).
Actinocyclus ralfsii (W. Smith) Ralfs, in Pritchard, *Hist. Infus.*, ed. 4, 835 (1861).
Actinocyclus ehrenbergii var. *ralfsii* (W. Smith) Hustedt, in Rabenhorst, *Krypt.-Flora*, **7** (1), 528 (1929).

Actinocyclus octonarius var. *sparsus* (Gregory) Hendey, *comb.nov.*

Eupodiscus sparsus Gregory, in *Trans. micr. Soc. Lond.*, N.S. **5**, 81 (1857).
Actinocyclus ralfsii var. *sparsus* (Gregory) Ralfs, in Pritchard, *l.c.*: 835 (1861).
Actinocyclus sparsus (Gregory) Rattray, in *J. Quekett micr. Cl.*, ser. 2, **4**, 170 (1890).
Actinocyclus ehrenbergii var. *sparsus* (Gregory) Hustedt, in Rabenhorst, *Krypt.-Flora*, **7** (1), 528 (1929).

Actinocyclus octonarius var. *tenellus* (Brébisson) Hendey, *comb.nov.*

Eupodiscus tenellus Brébisson, in *Mém. Soc. Sci. nat. Cherbourg*, **2**, 257 (1854).
Actinocyclus tenellus (Brébisson) Grunow, in *Hedwigia*, **6**, 31 (1867).
Actinocyclus ehrenbergii var. *tenellus* (Brébisson) Hustedt, in Rabenhorst, *Krypt.-Flora*, **7** (1), 530 (1929).

Navicula cyprinus var. *linearis* (Hustedt) Hendey, *var.nov.*

Navicula digito-radiata f. *linearis* Hustedt, in *Abh. naturw. Ver. Bremen*, **31**, 626 (1939).

Navicula cyprinus var. *rostrata* (Hustedt) Hendey, *comb.nov.*

Navicula digito-radiata var. *rostrata* Hustedt, in *Abh. naturw. Ver. Bremen*, **31**, 627 (1939).

Epithemia adnata var. *proboscidea* (Kützing) Hendey, *comb.nov.*

Epithemia proboscidea Kützing, *Die kies. Bacill.*, **35** (1844).
Epithemia zebra var. *proboscidea* (Kützing) Grunow, in *Verh. zool.-bot. Ges. Wien*, **12**, 329 (1862), '*proboscoidea*'.

ACKNOWLEDGMENTS

I am much indebted to the British Phycological Society for inviting me to undertake this work, and to numerous persons who have been kind enough to send me records of species. Particularly I wish to thank Dr C. L. Odam, Rev. R. Fraser Bastow, and Mr H. G. Barber for lists of species found in Wales and the west country. My especial thanks are due to Mr R. Ross of the British Museum (Natural History) for making available to me his unpublished work on the diatoms of Loch Sween which added numerous new

records to the flora, and for undertaking the onerous task of checking the proofs. I am indebted also to Dr Mary Parke, without whose encouragement this list never would have been completed.

Abbreviations of Authors' Names

Bail.	J. W. Bailey	Jörg.	E. G. Jörgensen
Bail. L. W.	L. W. Bailey	Kitt.	F. Kitton
Berg.	P. Bergon	Kütz.	F. T. Kützing
Berk.	M. J. Berkeley	Lagerst.	N. G. W. Lagerstedt
de Bréb.	A. de Brébisson	Laud.	H. S. Lauder
Brightw.	T. Brightwell	Lemm.	E. Lemmermann
Brockm.	C. Brockmann	Lindl.	J. Lindley
Carm.	D. Carmichael	Lyngb.	H. C. Lyngbye
Castr.	A. F. Castracane	Meun.	A. Meunier
Desmaz.	J. H. B. J. Desmazières	Müll.	O. Müller
Dick.	G. Dickie	Norm.	G. Norman
Dill.	L. W. Dillwyn	Ostenf.	C. H. Ostenfeld
Donk.	A. S. Donkin	Pant.	J. Pantocsek
D'Orb.	A. D'Orbigny	Pav.	J. Pavillard
Ehrenb.	C. G. Ehrenberg	Pell.	J. Pelletan
Eulen.	T. Eulenstein	Perag.	H. Peragallo
Feb.	H. Febiger	Pritch.	A. Pritchard
Freng.	J. Frenguelli	Rab.	L. Rabenhorst
Gaill.	M. Gaillon	Ratt.	J. Rattray
Greg.	W. Gregory	Schimp.	A. J. W. Schimper
Grev.	R. K. Greville	Schm.	A. Schmidt
Grun.	A. Grunow	Schm. M.	M. Schmidt
Har.	R. Harrison	Schum.	J. Schumann
Hass.	A. H. Hassall	Shadb.	G. Shadbolt
Heib.	P. A. C. Heiberg	Shrubs.	W. H. Shrubsole
Heid	H. Heiden	Smith	J. E. Smith
Hérib.	J. Héribaud	Stolt.	H. Stolterfoth
Hick.	W. J. Hickie	Temp.	J. Tempère
Hook.	J. Hooker	Trent.	J. F. Trentepohl
Hust.	F. Hustedt	Turp.	P. Turpin
Hutt.	W. Hutton	W. Sm.	William Smith
Jan.	C. Janisch	Wall.	G. C. Wallich

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

A Modification of Teorell's Method for Determining of Small Quantities of Ammonia

By **M. Buljan**

Arh. Hem. Farm., T. 23, 1951, pp. 119–22

The use of Teorell's method (*Biochem. Z.*, Bd. 248, 1932, p. 246) is not satisfactory, since subsequent titrations of the hypobromite solution by means of a solution of naphthyl-red do not produce reproducible results. This is due to the interference of oxidation products of naphthyl-red. A way of eliminating this difficulty is given.

If hypobromite is not added in sufficient quantity to destroy the ammonium salt to be determined, another difficulty is encountered. This is a change in the direction of the titration curve, and owing to this the consumed volume of naphthyl-red is no longer in a direct relation to the quantity of ammonia actually present. An attempt is made to explain the behaviour of hypobromite when there is a surplus of ammonia by the production of hyponitrous acid radical, which, in an acid medium, has oxidizing properties and decolorizes the solution of naphthyl-red.

In order to eliminate these difficulties encountered when applying the original method, it is proposed to use a solution of potassium-indigo-disulphonate for the titration of the surplus hypobromite in an alkaline medium, instead of a naphthyl-red solution in an acid medium, as recommended by Teorell. M.B.

Sulla Presenza di un Ormone d'Accrescimento in un Crostaceo Decapode, la *Lysmata seticaudata* Risso (On the Presence of a Growth Hormone in a Decapod Crustacean, *Lysmata seticaudata* Risso)

By **D. B. Carlisle** and **P. F. R. Dohrn**

Ric. sci. suppl., 1953, pp. 95–100

In this paper, read before the Convegno di genetica e di scienze affine, held in the Genetics Institute of the University of Naples in June 1952, the discovery of a moult-accelerating or growth hormone in a decapod crustacean was first announced. A fuller account was published later in the *Pubbl. Staz. zool. Napoli*, Vol. 24, 1953, pp. 69–83 (abstracted in Vol. 32, No. 2 of the *Journal*), which, however, appeared in print before this account. D.B.C.

STUDIES OF THE MESOGLOEA OF COELENTERATES.

I. HISTOLOGY AND CHEMICAL PROPERTIES

By **Garth Chapman**

Quart. J. micr. Sci., Vol. 94, 1953, pp. 155–76

Evidence of the collagenous nature of the mesogloea of coelenterates is provided by histological and chemical methods.

A description is given of the arrangement of the fibrous material forming the mesogloea in various medusae and in the actinians *Calliactis* and *Metridium*, where the crossed fibrillar structure is regarded as being determined by mechanical forces acting on the tissue.

The role of the mesogloea in the life of the animal is discussed; in particular its viscous-elastic properties are thought to be well adapted to its skeletal functions. G.C.

STUDIES ON THE MESOGLOEA OF COELENTERATES.

II. PHYSICAL PROPERTIES

By **Garth Chapman**

J. exp. Biol., Vol. 30, 1953, pp. 440–51

The physical properties of the isolated mesogloea of *Calliactis* and *Metridium*, which are mentioned in another paper (see above), are described in greater detail and the behaviour of the tissue on loading is recorded.

It is shown that the viscous-elastic properties of the body-wall which have been ascribed by previous authors to the muscles are the attributes of the mesogloea.

The thermal contraction of the mesogloea of *Calliactis* is compared with that of vertebrate collagen.

It is shown that the physical behaviour of the material is consonant with the crossed fibrillar collagenous nature of the mesogloea described in the other paper. G.C.

UPWELLING IN THE BENGUELA CURRENT

By **Ronald Currie**

Nature, Lond., Vol. 171, 1953, pp. 497–500

In 1950 the R.R.S. *William Scoresby* carried out two surveys of the Benguela Current, which is one of the major features of the circulation of the South Atlantic Ocean. This current is a region where cool, nutrient-rich subsurface water is upwelled to the surface and gives rise to a great production of planktonic plants and animals.

The observations indicate that the current is composed of a series of eddies, similar to those which Gunther had postulated in the Peru Coastal Current. In those eddies the upwelled water moving offshore converges sharply with interlocking tongues of warmer offshore water. In March upwelling was mainly in abeyance, but in September–October there was an active offshore transport of upwelled water. This is related to the more active coastal winds in the latter months. Analysis of the distribution of specific volumes of the water suggests that the mechanism of the upwelling is of a similar nature to that which occurs on the Californian coast.

The waters on the continental shelf have a very high inorganic phosphate content. It is suggested that this is partly due to local regeneration, as at some 'stations' the phosphate content was higher than that of the water being upwelled.

A deposit of diatomaceous mud, extending for some 400 miles along the coast, is populated with sulphate-reducing bacteria. It is overlain by water of a very low dissolved oxygen content. In March the oxygen depletion was much greater than in spring, and this is probably associated with the more quiescent state of the current in March. It is suggested that the O_2 depletion may to some degree be associated with mass mortalities of fish which frequently occur in the summer months. R.C.

MICRODETERMINATION OF PHOSPHORUS IN BIOLOGICAL MATERIAL

By **H. W. Harvey**

The Analyst, Vol. 78, 1953, pp. 110–14

An absorptiometric method for determining the phosphorus content of small quantities of material within the range of 1–70 μg of phosphorus has been devised and tested for precision. The material, collected by centrifuging, is decomposed in the centrifuge tube with sulphuric acid and hydrogen peroxide; pyrophosphoric acid is converted to orthophosphoric and any residual hydrogen peroxide decomposed with sulphite. The contents of the tube are diluted and the orthophosphoric converted to phosphomolybdic acid which is determined by the molybdenum blue method after controlled reduction with stannous chloride.

Experimental data are given to show the effect upon the blue colour formed due to (i) the concentration of stannous chloride, (ii) the temperature, (iii) acidity, and (iv) the time of reaction with stannous chloride. The effect of interfering substances is considered and experimental data are given which show the effect of traces of copper on the formation of the molybdenum blue.

H.W.H.

The Densities of some Common Aquatic Mollusca from Plymouth

By **A. G. Lowndes**

Ann. Mag. Nat. Hist., Ser. 12, Vol. 6, 1953, pp. 950–2

The density, sinking factor, and load carried when swimming or crawling are given for sixteen species of common Mollusca from the Plymouth district. The greatest density theoretically possible is something just short of that of aragonite, 2·95, while the lowest theoretical density is that of protoplasm, 1·05 (in the absence of fat).

Ocenebra erinacea L. attains a density of 2·07 while *Aplysia punctata* Cuvier has a density of 1·04. There is no apparent connexion between swimming ability and density. *Chlamys opercularis* (L.), whose density is 1·49, swims freely while *Aplysia* shows no sign of doing so. When *Chlamys opercularis* does swim it carries a load of 31 % of its weight. The load carried by a typical teleost with a swim bladder is less than 0·2 %. A.G.L.

Observations on certain Mechanical Properties of the Ligament of *Pecten*

By **E. R. Trueman**

J. exp. Biol., Vol. 30, 1953, pp. 453–67

The conditions of the opening and the closing of the valves of *Pecten maximus* and *Chlamys opercularis* have been illustrated by drawing stress-strain curves for the intact ligament and for isolated parts of the inner layer of the ligament. These curves form a hysteresis loop, the area enclosed in that of *Pecten* being markedly less than that of other lamellibranchs, indicating the greater efficiency of this ligament. This is attributed to the inner layer of the ligament, calcified uniformly in most lamellibranchs, but which in *Pecten* (and associated forms) has a large central non-calcified region. The modulus of elasticity in compression of this region is approximately one-seventh of that of the inner layer of *Ostrea* or *Lutraria*, which are calcified structures. Thus the inner layer of the ligament of *Pecten* has less resistance to compression but greater efficiency than that of most lamellibranchs. These properties are important in relation to swimming, in which the valves of *Pecten* open and close rapidly and frequently. E.R.T.

J. Mar. biol. Ass. U.K. (1954) 33, 565–85
Printed in Great Britain

OBSERVATIONS ON THE OPISTHOBRANCH MOLLUSC *ACTEON TORNATILIS* (L.).

By Vera Fretter, D.Sc.
Department of Zoology, Birkbeck College, University of London

and Alastair Graham, D.Sc.
Department of Zoology, University of Reading

(Text-figs. 1–9)

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Habits and External Features

Acteon tornatilis (L.), the most primitive British opisthobranch, may be collected in large numbers at Pendine Sands, Carmarthenshire, where, during early September, occasional individuals may be seen creeping over the shore near the water's edge at low tide. More conspicuous than the adult animals, however, at that time of the year are the gelatinous masses of spawn: these measure about 6 cm in length, are club-shaped and taper towards one end which is secured to a piece of weed, wood or stone, usually on the surface of the sand but often underneath. Sometimes molluscs are found with the egg mass trailing from the genital aperture: if these are followed they may be seen, when near an object projecting from the sand, to burrow a short distance, and, whilst the mollusc is out of sight, the last secretions are poured from the female opening and the mass fastened to the buried object, presumably by means of the foot.

During the ebb tide most of the specimens of *Acteon* which live in the area are not on the surface of the sand because they then tunnel through the upper 6 in. Their burrows open to the surface by a hole 4–5 mm in diameter, and, once this is realized, it is an easy matter to collect them. The method of burrowing may be followed in the laboratory. An individual placed on sand will creep over the surface leaving behind it a characteristic trail which is formed by the head lobes (i.e. expanded and flattened cephalic and labial tentacles) and by the widely expanded foot, the former lying in front of the latter (see Fig. 1). These structures act like a snow-plough, pushing aside the sand and heaping it on either side of the path; strong ciliary currents beat towards the free edges of the head and posteriorly over the sole of the foot to keep these surfaces clean, and the tentacles are reflexed over the edge of the shell so as to close off the mantle cavity. After creeping for a short distance over the surface the mollusc soon presses deeper into the sand and disappears from sight, the sand closing over behind it. What happens then may be seen if a tunnel be made against the side of an aquarium. The mollusc will travel vertically or diagonally downwards for an inch or two, then horizontally, and, sooner or later, will start towards the surface again. Its progress is slow. As it moves the burrow does not collapse behind it, for secretion from the head

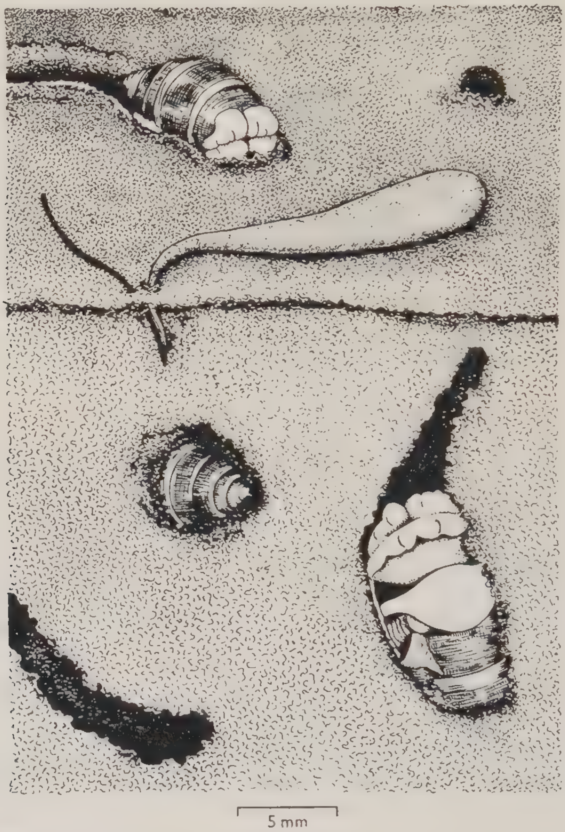

Fig. 1. *A. tornatilis* in its natural habitat. In the upper part of the drawing an animal is seen creeping over the surface of the sand, and the opening of a burrow is in the top right-hand corner. In the middle a mass of spawn lies on the surface attached to weed a little below. In the lower half of the drawing a mollusc is shown in its burrow. The exhalant siphon shows on its left.

lobes and pedal gland is pressed against the sand and agglutinates the particles. On reaching the surface the head is pressed back against the rim of the opening to make the edges firm with secretion and the animal may remain in this position for some time. Alternatively it may retreat, moving backwards as it goes, so that the head is kept facing the mouth of the burrow, and it may come to rest a few inches down with the semi-contracted foot holding firmly. The right margin of the foot overhangs the conspicuous exhalant siphon, which is partially protected, too, by the edges of the shell, and a slow, continuous current of water, apparently free from sand particles, passes out of the siphon. When the animal emerges fully from its shell this exhalant siphon comes to occupy the posterior corner of the mouth of the shell.

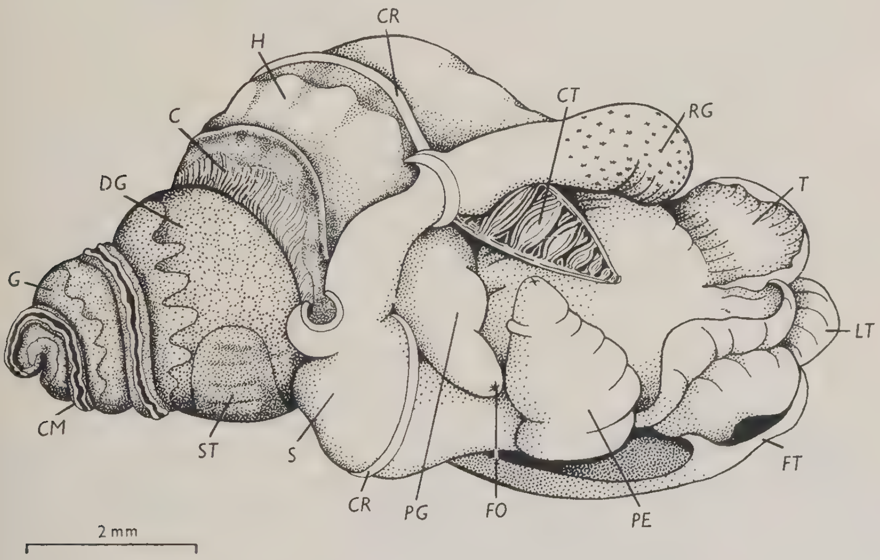

Fig. 2. *A. tornatilis* with shell removed. For explanation of lettering see p. 585.

Some of the primitive features of *Acteon* may be correlated with the large size of the shell, which permits the animal to withdraw completely and seal itself with an operculum. The body whorl is deep, its aperture an elongated slit along the right side with a length equal to about two thirds the height of the shell. This may be correlated with the fact that the mantle cavity of *Acteon* is at least as large as in any other opisthobranch and resembles that of a prosobranch in having to accommodate such structures as a large penis (Fig. 2, *PE*) which cannot be invaginated, and pallial genital ducts (*PG*) which in many gastropods have sunk into the haemocoel. The shape of the cavity differs from that of the typical prosobranch in two important respects: the opening, though large and anterior, is markedly twisted to the right so that there is no opening left of the mid-line, where the gill is attached; and the cavity is drawn out posteriorly into a narrow extension (*CM*) which runs separately alongside the main spiral of the visceral hump almost or quite to its apex.

The posterior extension of the mantle cavity lies right of the gill, but left of the rectum and genital tract, and is, therefore, a derivative of the hypobranchial region. Along it run two grooved and ciliated ridges (*CR*), both of which lead to the free edge of the mantle skirt anteriorly. The grooves were not described by previous workers. The left of these, the larger of the two, lies parallel to the ctenidium and is placed just right of the mid-line; the right lies left of the rectum and leads on to the expanded flap of pallial tissue used as an exhalant siphon (*S*). Glandular tissue intermixed with ciliated cells lines the wall along and between these ridges and represents the hypobranchial gland. The two ridges and intervening tissue separate from the main mantle cavity as the caecum which coils round the visceral hump. The anus lies in the innermost part of the main chamber.

In the caecum the grooved ridges come to lie opposite one another and the whole organ is triangular in cross-section (Fig. 4A), the base resting against the upper side of the whorls of the visceral hump, not flattened as described by Perrier & Fischer (1911). The space between the ridges on the outer side (the posterior or sutural space of Perrier & Fischer) leads on to the space between the ridges at the edge of the mantle skirt; that between them on the inner side (the anterior space of Perrier & Fischer) leads, at the mouth of the caecum, to the base of the gill on the left, to the rectum on the right.

Between the epithelium lining the cavity of the caecum and the mantle lie blood spaces (Fig. 4B, *V*) criss-crossed by slender strands of muscle. Two main channels run directly under the ciliated ridges and there is here a band of longitudinal muscle fibres. In the vessels underneath the ridges blood cells packed with granules (*AM*) are abundant and these may often be seen in the process of passing into the groove. The epithelium which lies at its base is often, in fact, much distorted by their numbers.

The glands of the caecum are of two sorts, one a 'mucous' cell (*MC*), secreting mucoitin sulphuric acid, the other (*GC*) producing granules of a substance staining intensely with iron haematoxylin. Both are of types commonly encountered in the hypobranchial gland of other gastropods.

Ciliary currents (Fig. 3) run across the ridges into the posterior space of the caecum, where they set in an outward direction so that particulate matter (including extruded blood cells) is swept to the region of the exhalant siphonal fold on to which currents from the anus and surrounding parts also converge. A compensating ingoing current travels up the anterior space of the caecum so that a circulation of water is maintained.

The edge of the mantle skirt in *Acteon*, in its left half, is greatly thickened by the presence of elaborate glands called by Pelseneer (1894) the semilunar gland. These form a belt, parallel to the edge, which has a pitted aspect, each depression being the mouth of a compound tubular or flask-shaped gland. If

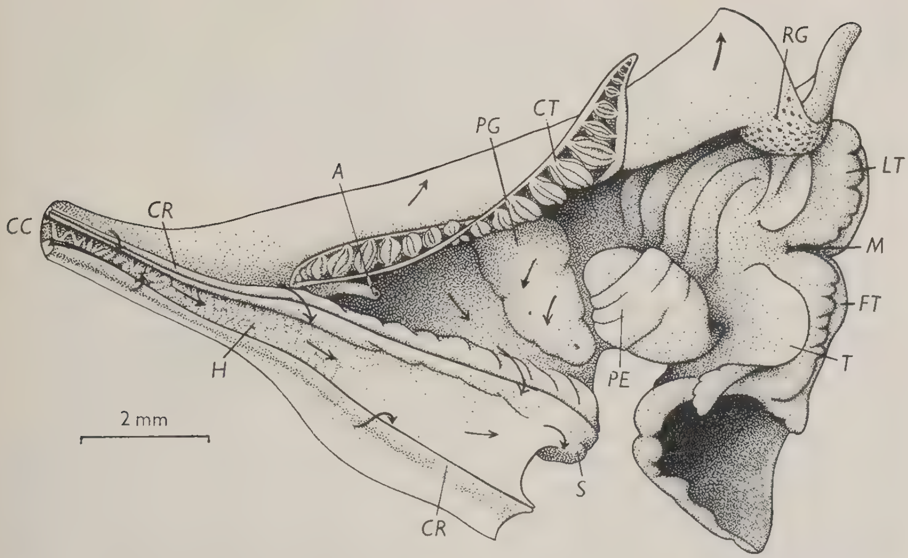
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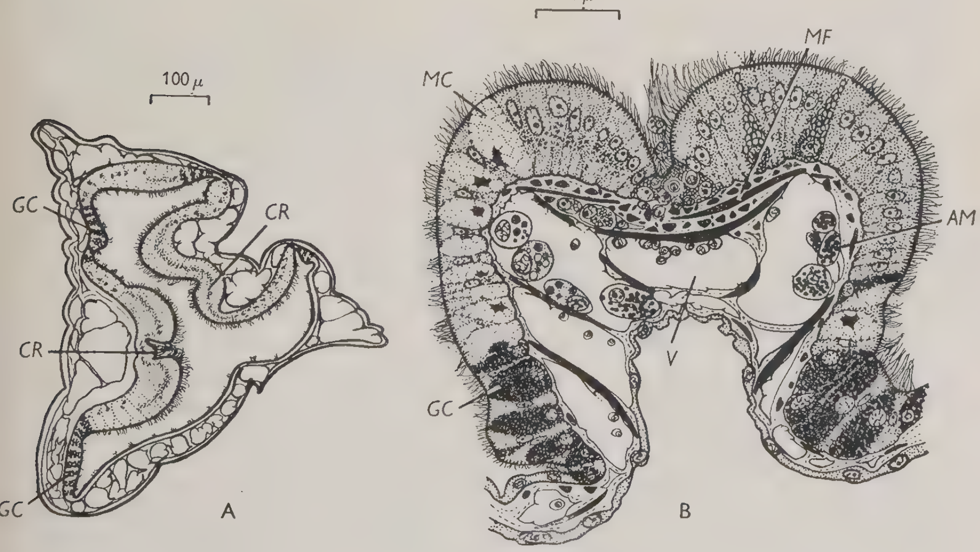
Fig. 3. *A. tornatilis.* Mantle cavity and base of caecum opened by a longitudinal slit to the right of the ctenidial axis. Arrows show direction of ciliary currents. For explanation of lettering see p. 585.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MF
100 μ
MC
GC
CR
AM
CR
V
GC
GC
A
B

Fig. 4. *A. tornatilis.* A: transverse section of the caecum; B, transverse section of ciliated ridge of the caecum. For explanation of lettering see p. 585.

a living specimen of *Acteon* be irritated in this or neighbouring regions small, white, spirally coiled threads are discharged from the mouths of the glands. Experiment shows that the effect of this secretion on living animals is toxic so that the glands must be regarded as repugnatorial. The histology of the gland has been adequately described by Perrier & Fischer (1911).

The Alimentary Canal

The alimentary tract of *Acteon* opens at the mouth placed anteriorly on the head between two lips formed from the bases of right and left labial tentacles. Dorsal to these the cephalic tentacles lie as forwardly curving flattened sheets of tissue. The mouth leads into a short oral tube running back to the buccal cavity. Ventro-laterally, just within the mouth, are two papillae on which open ducts from oral glands that are indicated in Guiart's plate (1901), but the existence of which Fretter (1937) denied. Their secretion is not mucous, despite the allegation of Gabe & Prenant (1952). The lining of the oral tube here is a low epithelium covered with a thin cuticle and it is flung into two or three irregular folds. More posteriorly the oral tube is narrowed to a dorso-ventral slit passing between two bulges, one in each lateral wall. These are muscular and the epithelium over them bears a cuticle made of small oval plates each with a denticulate edge. These are the structures described by Fretter (1937) as the radula, but they are in fact the jaws, as shown by Gabe & Prenant (1953), and her criticism of Sars's (1878) description is not warranted.

Still deeper in the animal's head the gut expands once more to form the chamber of the buccal cavity. On the floor of this lies the rather small odontophore, deeply cleft on its dorsal side by a longitudinal groove. Over its dorsal and lateral surfaces is spread the radula, looking much like a cuticle raised slightly in a regular pattern, so slight is the development of the teeth. No division into different kinds is possible—indeed it is difficult to recognize the structures as teeth at all. At the posterior end the radula originates in the merest fold of the wall of the gut; it is hardly possible to regard it as a separate radular sac, although it has the correct morphological relationships of such.

The buccal mass contains no cartilages, but is largely composed of connective tissue with deposits of calcium salts and glycogen (Gabe & Prenant, 1952). Its muscular equipment, too, is much weaker than is usual and amounts to little more than a thin coat of reticulating fibres in its superficial parts. There are no mucous glands here (Fretter, 1937).

The floor and the sides of the buccal cavity are covered with a glandular epithelium and are, in consequence, white in colour. Into the cavity, dorsally, discharge two salivary glands, the histological structure of which has already been described fully by Fretter (1937).

The oesophagus shows no trace of the glandular equipment of a prosobranch, nor of torsion. It is a simple tube, with many parallel folds on its

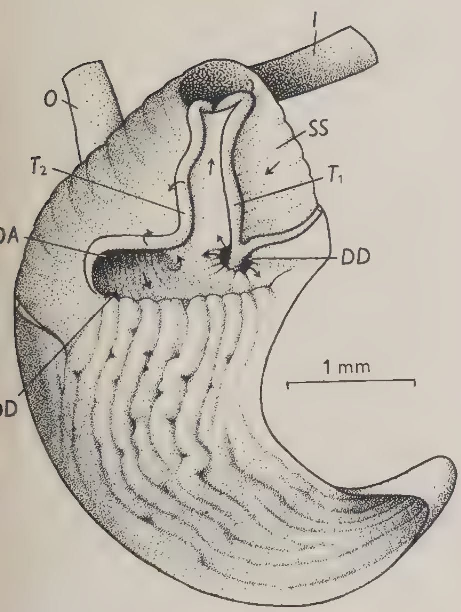
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Fig. 5.

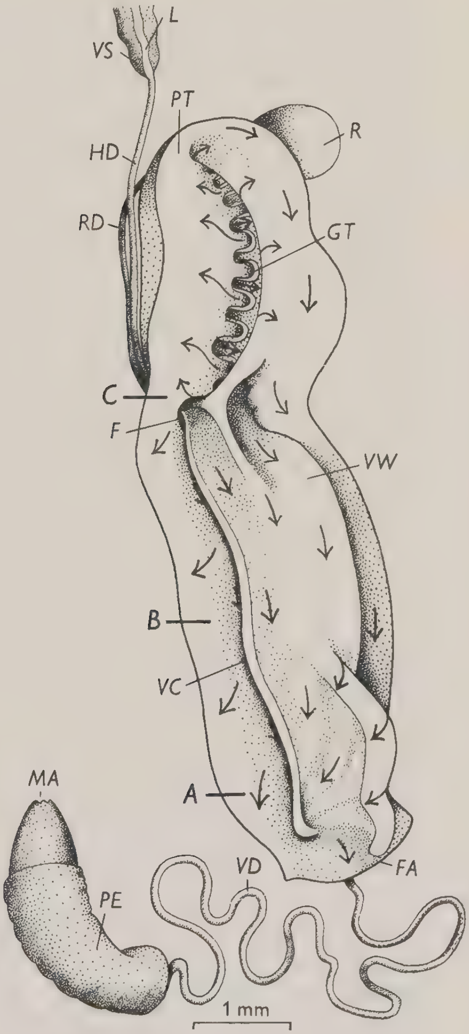
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Fig. 6.

Fig. 5. *A. tornatilis.* Stomach opened dorsally. Arrows show direction of ciliary currents. For explanation of lettering see p. 585.

Fig. 6. *A. tornatilis.* The genital ducts. The mucous gland has been opened along its length. Arrows show the direction of ciliary currents. *A, B, C,* mark positions of sections diagrammatically represented in Fig. 7.

inner epithelium, which leads back to the stomach (Fig. 5), a part of the gut not adequately described before. It is, like the oesophagus, much simpler than in prosobranchs and shows the characteristic tendency of the opisthobranch to have the oesophageal and intestinal apertures close together at the lower or morphologically posterior end. The process of simplification, however, has not proceeded as far as in many other opisthobranchs and it is still possible to distinguish major (T_1) and minor (T_2) typhlosoles related to the style sac region (*SS*) and to the two ducts of the digestive gland (*DD*). The bulk of the organ extends as a simple sac up the visceral hump. Ciliary currents occur on the walls near the openings of the oesphagus and the digestive gland and in the style sac, but the sac-like part is not ciliated except in small strips. The cells bear, instead, a fine cuticle.

There is a tendency for the posterior part of the oesphagus and the initial part of the intestine to enlarge, a process carried much farther in the basommatophoran pulmonates (Heidermanns, 1924).

The intestine is short and takes a sinuous course from left to right to open by the anus. Its epithelium is columnar and ciliated and contains mucous cells. The digestive gland is large and bilobed and occupies most of the upper whorls of the visceral hump. Its histology is described by Fretter (1937) and Gabe & Prenant (1952).

It is difficult to be certain of what the animals eat. The most conspicuous contents of the gut are sand grains and black spherules of excretory matter derived from excretory cells in the digestive gland. There is also fragmentary material of animal origin, but not in sufficiently large pieces to allow of identification. The molluscs have, unfortunately, not been seen in the process of feeding so that, although their jaws and radula suggest the possibility of their raking small animals into the gut, precisely what animals it is that might so be treated remains doubtful.

THE REPRODUCTIVE SYSTEM

A. tornatilis is a protandrous hermaphrodite. The course of the genital ducts has been described by Bouvier (1893), Pelseneer (1893) and Guiart (1901), and on these descriptions Fretter (1946) has based a comparison with the genital ducts of other gastropods. Pelseneer's figure of the ducts is adopted by Guiart. It shows the little hermaphrodite duct passing forwards from the gonad and joining the duct from a copulatory pouch in which sperms from another individual are stored. The common duct then leads to a large glandular structure associated with the female system, and comprising an albumen gland and a mucous gland. It is stated that on their way through this the outgoing sperm are confined to a gutter between two folds which, near the opening to the mantle cavity, close to form a vas deferens running forwards to the penis. Near the divergence of the vas deferens the female duct opens to the exterior.

This description may have been put together from a study of dissections (Fig. 6) for when the glandular pallial oviduct is opened by a longitudinal slit, there can be seen a gutter (*VC*) which leads from near the opening of the little hermaphrodite duct (*HD*) to the point of origin of the vas deferens (*VD*). Indeed this channel and the deferent duct appear to be confluent. Sections, however, show that this is not so. The longitudinal gutter, a ciliated and muscular channel, where gland cells are rare, arises anteriorly (without connexion with the vas deferens) near the genital aperture (*FA*) and leads posteriorly to the duct (*RD*) of the copulatory pouch or receptaculum seminis (*R*). It receives this duct, and, immediately beyond this point, opens to a small pouch which is the focal area of the female reproductive system since it also communicates with the hermaphrodite gland and with the albumen gland.

In addition to this connexion the little hermaphrodite duct, which bifurcates at its lower end, connects to a prostate gland (*P*), the existence of which was not known to Pelseneer, Guiart, or to Gabe & Prenant (1952). The prostate lies in the same position as the glandular oviduct, for the most part beneath it (*VW*), the two being so intimately connected that it is difficult to separate them. Anteriorly it is the *prostate* that narrows to the vas deferens (Figs. 6, 7, *VD*) which runs forward to the penis (Fig. 6, *PE*) on the right of the head. Thus from the point of bifurcation of the little hermaphrodite duct near its lower end male and female channels are entirely separated.

The structure of the hermaphrodite system may now be followed in greater detail. The description is based on animals which were collected during early September, whilst spawning.

The tubules of the gonad are hermaphrodite, each giving rise to eggs and sperm. The little hermaphrodite duct into which the gonad opens is ciliated throughout its course and has a moderately thick coat of circular and longitudinal muscle fibres. On functional and histological grounds it is divisible into three regions: short proximal and distal parts for conduction only, and an intermediate one which is swollen with unorientated sperm even when eggs are passing to the oviduct. The entrance to this seminal vesicle (Fig. 6, *VS*) is by way of a muscular papilla, and there is a valve which controls the passage through the duct near the glandular pallial ducts. Dissections of animals which have just spawned show the dilated vesicle as a broad duct, brown in colour, except for a longitudinal streak (*L*) marking a thin ciliated tract on the wall through which can be seen the iridescence of the underlying sperm. The streak has the same simple ciliated epithelium as the conducting regions of the duct; elsewhere the histology is more complex. Bordering the ciliated tract are gland cells with exceptionally large nuclei and containing protein spherules. The glands are placed between ciliated cells and do not seem to be present in younger animals in which only the male system is mature. Covering a much greater area on the wall opposite the ciliated tract are cells with fine, short cilia and vacuolated cytoplasm, the vacuoles containing spherules. The important feature of these cells is that they can ingest spermatozoa; within the cytoplasm ingested gametes lose their identity and are apparently digested. Absorption of effete sperm by the epithelium of the vesicula seminalis has been recorded for *Littorina* (Linke, 1933), the Stenoglossa (Fretter, 1941) and the tectibranch *Onchidella celtica* (Fretter, 1943). The two branches of the hermaphrodite duct, after its bifurcation into male and female channels, embrace the duct of the receptaculum seminis as it opens to the ventral channel.

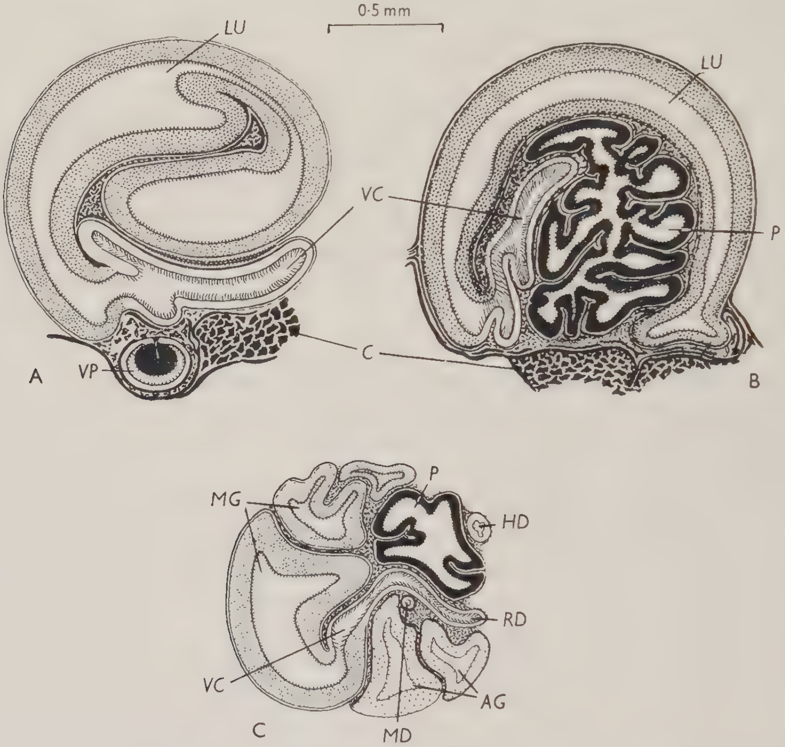

Fig. 7. *A. tornatilis*. Diagrammatic transverse sections through pallial genital ducts at levels indicated in Fig. 6. For explanation of lettering see p. 585.

The prostate (*P*) constitutes the broad pallial region of the male duct. Here the epithelium is thrown into deep longitudinal folds and it has, therefore, a large secreting area. The gland cells are all of one sort, each containing a number of protein spherules, and they alternate with small ciliated cells. This part of the male duct lies immediately above the columellar muscle and under the ventral wall of the oviduct (Figs. 6, 7, *VW*), to which it is joined by a sheet of connective tissue. Anteriorly, near the origin of the vas deferens, the prostate narrows, and its ventral wall becomes a strongly ciliated channel without glands. In this transitional region the typical secreting cells of the upper portion are confined to the dorsal wall where they form a ridge projecting deeply into the lumen (*VP*). The deferent duct (*VD*), which passes anteriorly, has the same diameter throughout until it approaches the tip of the penis where it contracts. It is ciliated and not glandular. The penis, which it penetrates, is not invaginable as in other opisthobranchs and is unusually large. When the penis is at rest the duct coils in the haemocoel at its base and in the blood space which lies centrally within it. It is excessively muscular. The tip of the penis forms a glans kept turgid by a core of connective tissue; when the organ is relaxed the tip is slightly sunk into the surrounding tissues which form a shallow fold around it like a prepuce. The vas deferens has a straight course through the connective tissue of the glans.

The female system has characteristic tectibranch features which concern, among other things, the histology of its glands and the relative position of its parts. The gland cells alternate with ciliated cells and are never subepithelial in position. Most of them secrete mucus. The secreting areas of the oviduct comprise two distinct masses: a posterior one (*PT*) referred to by previous workers as the albumen gland and a larger and more anterior one, the mucous gland. The lumen of the former is complex, that of the latter is a simple channel. The ventral ciliated gutter (*VC*) is folded off from the secreting epithelium and it is presumably along this that the penis lies to reach the duct of the receptaculum. The penis, when extended, can attain a length which would permit this, and the channel is broad enough to contain it.

The posterior part of the pallial oviduct, the so-called albumen gland, is a composite structure: some of its tubules open into a centrally placed space which almost certainly functions as a fertilization chamber, whereas the remainder communicate with the upper part of the mucous gland since this extends alongside the albumen gland as a straight channel, reduced in size, as far as the posterior end of the oviduct. In sections the true albumen gland can be distinguished from the surrounding mucous tunic on account of the different histological features of the two. The mucous gland has a variety of secreting cells in its epithelium, though the kind which covers most of its surface is the mucous cell filled with uniform spherules. There are, however two places where the gland cells do not produce mucus. These lie along the ventral wall, where they form a narrow strip bordering the ciliated channels and anteriorly, in the epithelium which precedes the genital papilla and spread posteriorly for a short distance along the lateral walls of the gland. The secretion from this anterior end of the mucous gland is more viscid than that produced posteriorly.

Near the genital aperture the glandular epithelium stops rather abruptly, and the ventral channel enlarges to form the small papilla on which the female opening is placed. The epithelium here is ciliated as in the channel.

The plan of the various parts of the female system suggests that the site of fertilization of the eggs is at the confluence of the albumen and mucous glands. Here is the pouch, already referred to, which receives the female branch of the hermaphrodite duct and also secretion from the albumen gland; it leads directly into that part of the mucous gland into which the receptacular duct opens. It has already been noted by Bouvier (1893) dealing with *Acteon solidulus* that the receptacular duct may be filled with sperm like the receptaculum itself. In *A. tornatilis*, when spawning, sperm are orientated along the duct, dilating it considerably if they are present in large numbers, but they do not appear to occur in the receptaculum. That pouch is filled with a yellowish brown, viscous fluid, most of which responds to stains for mucus in the same way as secretion from the female tract whilst the other contents stain like prostatic fluid. From these facts it may be deduced that the receptaculum and its duct have a dual function as in the stenoglossans *Nucella* and *Buccinum* (Fretter, 1941) where it acts as a storeplace for sperm and, in distally placed outgrowths, for the destruction of superfluous sperm and granules of yolk. There is, however, no obvious absorption in *Acteon*.

The mouth of the receptaculum and its duct bear a ciliated epithelium; in the pouch, on the other hand, cells with large nuclei and cytoplasm filled with spherules in vacuoles form a columnar epithelium.

The embryos contained in each mass of spawn are individually supplied with albumen and may be seen rotating in this when they have reached the veliger stage. A membrane separates the albumen from the common jelly in which all are embedded, and a more viscid outer layer gives shape to the spawn and forms the cord for attachment. Presumably each egg is fertilized and enclosed in albumen within the fertilization chamber, and is then freed to the general cavity of the mucous gland. Dissection of an animal which is spawning shows that there are large quantities of secretion filling this gland prior to the entry of the eggs, and also shows how the eggs, enclosed in albumen, are forced into this secretion. The spawn mass is retained in the mucous gland until it is complete. The rounded distal end is the first to emerge from the genital aperture, the stalk for attachment leaving last. The glands in the part of the oviduct nearest the mouth produce much of the fluid of which the stalk is composed. The spawn swells when exposed to sea water and superficially resembles that of other tectibranchs such as *Haminea* and *Philine*. As in these genera the young of *Acteon* escape at the veliger stage.

DISCUSSION

The earliest fossil representatives of *Acteon* are shells of *Acteon* (= *Cylindrites*) *acutus* (Sow.) from the calcareous portion of the Great Oolite (Bathonian). *A. tornatilis* does not appear until the Miocene when it occurs in deposits in France, Austria and northern Italy. These may well have been the most ancient of opisthobranchs and have had a considerable range in European waters. A century ago Forbes & Hanley (1853) gave the distribution of *A. tornatilis* as throughout the British Isles, with a general range from Norway to the Mediterranean. How abundant the animal then was the authors do not say, but our general conception of the distribution of *Acteon* to-day is that of a rather uncommon animal living in sand at L.W.S.T. and to a depth of sixty or more fathoms, since only very occasional specimens are dredged at Plymouth, Port Erin or Millport. Such infrequent capture, however, may have little meaning since an aquarium known to contain many specimens may exhibit few on account of their burrowing habit; and at Pendine sands the numbers obtained without digging give no idea of their real frequency. They are, in fact, abundant at this spot and presumably in similar habitats throughout Britain.

The habit of burrowing is shared with other opisthobranchs such as *Haminea*, *Scaphander* and *Philine*. As adaptations for this the foot is powerful and the tentacles form lobes round the mouth for shovelling sand away; the cephalic tentacles become flat and spoon-shaped and are used for the same purpose and for blocking the entrance to the mantle cavity, and the eyes sink into the tissues of the head. It may be partly in connexion with this habit, too, that these animals (along with *Scaphander* and *Acera*) have evolved the pallial caecum which runs up the spiral of the shell alongside the visceral hump. The most obvious function for such a structure would be to aid respiration, not only by itself providing a respiratory surface, but also by leading a current of water to the neighbourhood of the upper parts of the visceral hump. It may be noted that the gill is small and is built on the usual opisthobranch rather than upon the usual prosobranch pattern. This may well be a less efficient type of gill since in most cases where it occurs the body of the mollusc is naked or only partially covered by shell so that secondary respiratory surfaces are large. *Acteon*, however, is a type of opisthobranch in which the body may be completely withdrawn into a shell and in which a great deal of the surface of the body is covered even at maximal expansion. In these circumstances accessory respiratory surfaces within, or mechanisms for accelerating the flow of water through, the mantle cavity may be necessary and the caecum may be such a device. In the pyramidellids (Fretter & Graham, 1949), in *Omalogyra* and *Rissoella* (Fretter, 1948) there is no gill at all, and it is fundamentally the same method which is used in each of these animals to compensate for its absence—longitudinal ridges of ciliated epithelium in the neighbourhood of the hypobranchial gland, which produce a strong exhalant current. These animals, therefore, differ from most molluscs in replacing an inhalant by an exhalant force as the main driving power of their respiratory water-current in the mantle cavity. This strong exhalant stream is exploited to some extent in all these animals for the evacuation of excretory matter contained in amoebocytes. These abound in the blood vessels of the pallial caecum in *Acteon*, from which they escape to the ciliated grooves by diapedesis and are swept down to the exhalant siphon. A similar extrusion of blood cells laden with excreta was noted by Fretter & Graham (1949) in the pyramidellids. It may partly be due to the strength of the current, too, that the anus has retained its primitive position in the innermost part of the mantle cavity instead of extending to near the edge of the mantle skirt. The similarity of these molluscs as regards their production of pallial water currents offers further evidence that pyramidellids and *Omalogyra* and *Rissoella* are opisthobranchs.

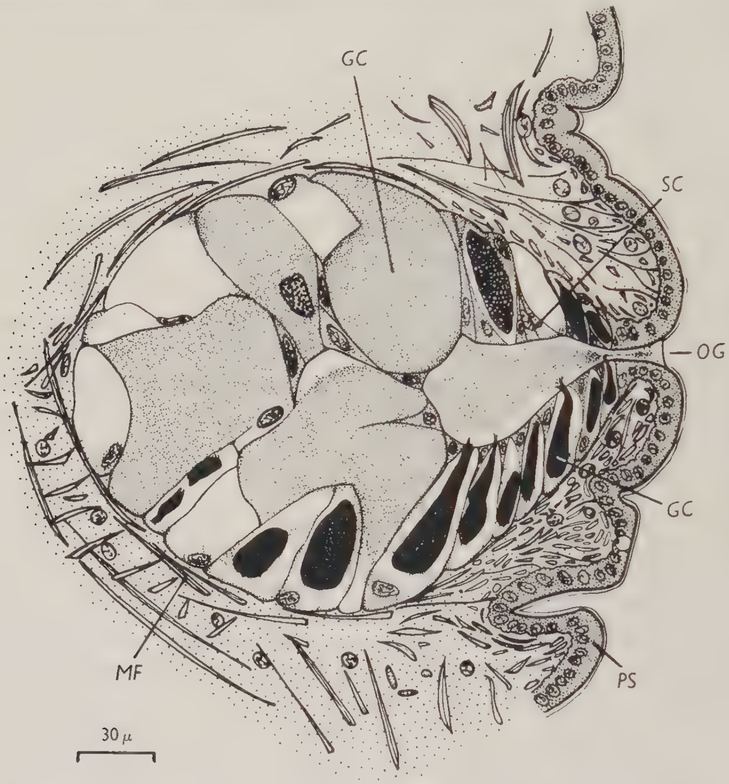

Fig. 8. *Siphonaria* sp. Vertical section of lateral pedal gland. For explanation of lettering see p. 585.

Like many opisthobranchs *Acteon* has a number of special glands associated with the mantle cavity and the mantle itself. When irritated it liberates a purple secretion from the hypobranchial gland and toxic secretions from the repugnatorial glands on the mantle skirt. Glands of almost identical histological nature may be found at the pallial edge or on the sides of the foot of many nudibranchs, opisthobranchs and marine pulmonates. Fig. 8 illustrates such a gland in *Siphonaria*. *Onchidella celtica* (Joyeux-Laffuie, 1882; von Wissel, 1898; Fretter, 1943) possesses very similar ones except that they discharge by longer ducts. Many limpet-like prosobranchs, too, in which the conical shape of the shell and the absence of operculum prevent the animal from shutting up entirely when disturbed, possess similar or even more elaborate glands. These have been described for *Calyptraea* and *Crepidula* by Graham (1954), but perhaps the most complex arrangement is to be met in *Patelloida virginea.* If the mantle edge of this animal be examined it will be found to be thickly set with a series of unicellular glands round its entire length (Fig. 9). The margin of the mantle also carries, at regular intervals, groups of special cells carrying long, motionless cilia set on slight projections. Even when the mollusc is closely pressed to the underlying rock these ciliated structures project a little beyond the edge of the shell. There are three types of gland cell. The first, the most conspicuous and the commonest, is a flask-shaped gland, bright white in colour, the body of the flask set deep in the pallial tissue and communicating with the edge by a long duct. The body of each of these cells is surrounded by a basketwork of muscle fibres and the cytoplasm is granular. On contraction of the muscles threads of white secretion are squeezed into the water where they retain their identity for some time. Exactly similar secretion comes from the glands in *Acteon*, *Onchidella* and the calyptraeids, and experiment by one of us (V.F.) has shown that this is rapidly toxic to small animals.

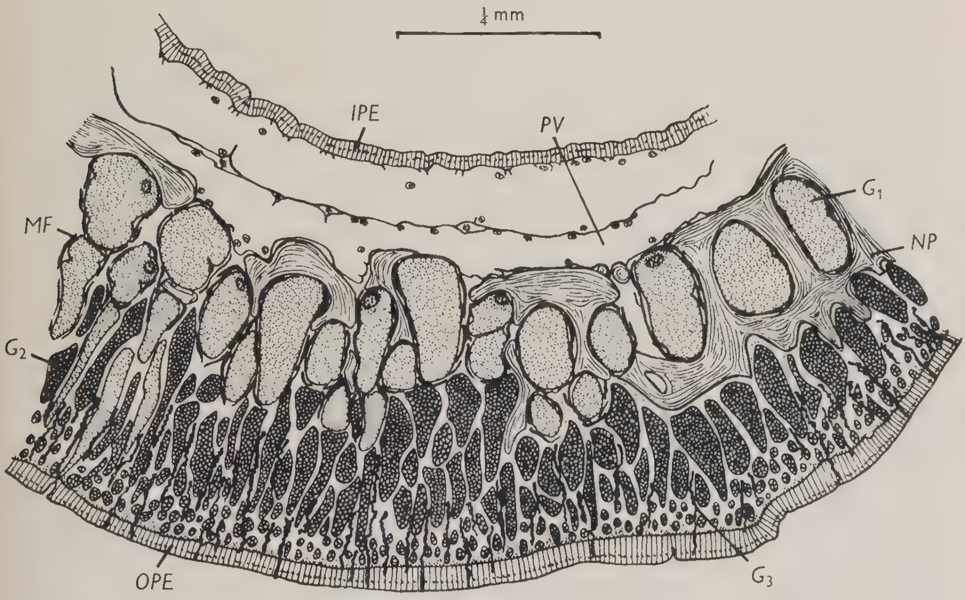

Fig. 9. *Patelloida virginea.* Tangential section of part of edge of the mantle skirt showing the nerve plexus, gland cells and muscle fibres around gland cells. The sensory organs on the outer pallial epithelium do not occur at this level. For explanation of lettering see p. 585.

The two other types of gland in *Patelloida* lie in banks successively nearer the mantle edge and they have correspondingly shorter ducts. The second is also a large, pear-shaped gland, but it lacks the muscular coat of the first type, its cytoplasm is homogeneous and uncoloured and its secretion appears to disperse. The third type is a bright red in a living mollusc and the gland is short and tubular. All these glands, but the first type in particular, are surrounded by a great plexus of nerves connected to the main pallial nerve, which also involves the groups of sensory cilia set on the mantle edge. It seems likely that here is a local arrangement of sense organs (the ciliated cells), nerve centres (the plexus) and effectors (the gland cells and their muscle coat) which provides a defensive mechanism protecting the mollusc against predators. In general, it may be said that wherever loss of shell or operculum deprives the animal of mechanical protection, chemical protection has appeared as a substitute. Protective devices of this kind, however, are noticeably absent in such well-known limpets as *Patella*. This may well be due to their ability to live on such exposed surfaces as would protect them against any potential predator on which secretions of this kind would have any effect.

In the majority of prosobranch gastropods the sexes are separate, fertilization is internal and the eggs are laid in capsules which are secreted by the pallial region of the oviduct. This region traffics in both sperm and ova: although its elaborations are apparently mainly concerned with the production of secretions for capsules, they also comprise well-defined tracts and pouches for the manipulation of sperm received during copulation. In *Littorina* and *Nucella*, and in other genera, the bursa copulatrix receives the seminal fluid from the penis of the partner and a ciliated groove, free from glands, is used by the sperm to travel the length of the pallial oviduct and reach the receptaculum seminis. The receptaculum is at the inner end of this duct.

In the opisthobranchs hermaphroditism is the rule. A hermaphrodite gland replaces the unisexual gonad, and the ducts which pass its products to the exterior must deal with outgoing as well as incoming spermatozoa, and produce the gelatinous secretions which characterize the spawn. In detorted gastropods, both pulmonates and opisthobranchs, the pallial duct has separated from the body-wall which restricted its further evolution in the prosobranchs and, freed to the haemocoel, it attains a further degree of complexity and a relatively larger size. The tectibranch *Acteon tornatilis* is an exception. Although it has an assemblage of opisthobranch characters which include hermaphroditism, its genital ducts are still linked to the musculature of the body-wall and have a straight course from gonad to genital aperture. In some tectibranchs such as *Philine*, *Scaphander* (Lloyd, 1952) and *Aplysia* (Eales, 1921) the plan of the hermaphrodite duct is similar to that of the female prosobranch in that it is single from gonad to genital aperture, and is simply divided by longitudinal ridges into sperm-conducting and egg-conducting grooves; whilst the groove which conducts sperm to the exterior passes forwards from the female opening to the penis on the right side of the head, giving an open vas deferens—a condition which may be primitive for the prosobranchs (Fretter, 1946) as well as the opisthobranchs. *Acteon* is not primitive in this respect since from its origin at the distal end of the gonadial duct the vas deferens is closed, and, throughout its course, is separated from the oviduct. The vas deferens leads to the penis which is, of course, unaffected by detorsion. In *Acteon* the mantle cavity is large and, as in prosobranchs, offers protection to the penis, whereas in other tectibranchs with a reduced mantle cavity, the penis, when not in use, lies retracted in a sheath sunk into the haemocoel.

Perhaps it is on account of the separation of pallial vas deferens and oviduct, and the possession of a penis of exceptional size, that the arrangement of sperm pouches in the female tract of *Acteon* differs from that of *Philine* and *Scaphander* in which the prosobranch plan is adhered to. In these two genera the homologue of the bursa copulatrix of prosobranchs is not far from the female opening, and is characterized by the possession of a long, muscular duct and pigmented contents of the pouch. If copulation be reciprocal the insertion of the penis into the duct, which receives the seminal fluid, would not block the passage of the hermaphrodite tract nor interfere with the flow of spermatozoa to the recipient's own penis. A deeper insertion of the penis to the vicinity of the receptaculum might well do so, however. Now in *Acteon* male and female tracts are separate ducts and the penis is long enough to extend to the inner end of the pallial oviduct: a bursa near the female opening is not developed and it is assumed that the penis uses the ventral channel of the oviduct to deposit sperm in the duct of the receptaculum, where they may be found orientated soon after copulation. In structure and in function the receptaculum resembles the bursa and not the receptaculum of *Philine* and *Scaphander*—the duct long and muscular, the pouch spherical and conspicuous on account of its size and pigmented contents. These comprise prostatic fluid received during copulation and sometimes other secretions from the genital ducts. Secretion is apparently also produced by the epithelium of the pouch itself: this may agglutinate the debris or cause the breakdown of the proteins (Lloyd, 1952). The ultimate fate of the contents is unknown: it may be that they are passed into the surrounding haemocoel and so utilized by the animal. A receptaculum and a bursa (the spermatheca of Eales) are developed in *Aplysia*, the former receiving sperm directly from the penis, the latter accumulating unwanted material introduced with the sperm and perhaps absorbing it or discharging it to the exterior (Eales, 1921).

As in other tectibranchs like *Onchidella* (Fretter, 1943), *Aplysia* (Eales, 1921), *Philine* and *Scaphander* (Lloyd, 1952) the albumen gland is an appendage of the female tract: this contrasts with prosobranchs where the eggs traverse the cavity of the gland.

In a comparison between the genital ducts of prosobranchs and opisthobranchs Fretter (1946) regarded the arrangements of the ducts in *Acteon* as showing remarkable agreement with those of the female *Littorina* and the Stenoglossa. The comparison was based on Guiart's (1901) description of *Acteon tornatilis* in which the prostatic region of the vas deferens underneath the pallial oviduct was not mentioned and the ventral channel of the oviduct was regarded as the vas deferens. Now that another investigation of that species has been made it appears that the reproductive system displays a mixture of primitive and advanced characters. The former, which are shared with the prosobranchs, relate to the large and uninvaginable penis and the association of the pallial ducts with the body-wall. The latter concern the loss of the large hermaphrodite duct with the separation of male and female channels, a character which is unusual in the tectibranchs, though found in higher opisthobranchs.

The alimentary tract shows, in general, an apparent simplicity, even the radular structure having a superficial suggestion of extreme primitiveness. When, however, the gut is compared with that of a prosobranch the loss of all trace of oesophageal glands, of all signs of torsion, the complex histology of the salivary and digestive glands and the modification of the stomach indicate that the simplicity must be secondary and the radular structure advanced in relation to a (presumably) carnivorous way of life. The position of the anus is, in fact, the sole genuinely primitive character which the gut appears to possess, and there is no very obvious reason why that might not also be secondary.

Acteon, therefore, is a gastropod with a medley of prosobranch and opisthobranch characters. The external facies is chiefly prosobranch, with its emphasis on shell and operculum, its large mantle cavity still facing well forward and the uninvaginable penis. Apart from the gill, which is opisthobranch in organization, the main departures from the usual prosobranch appearance might well be directly due to adaptation for burrowing.

So far as internal anatomy is concerned the nervous system, with its streptoneury and lack of fusion of ganglia, is frankly prosobranch in plan; and so is the auricle lying anterior to the ventricle. The reproductive system, on the other hand, shows a mixture of characters and the gut is distinctly advanced.

Acteon and the Acteonidae, in view of this mixture of characters, must always occupy a debatable position in any clear-cut classification. There is, however, a gentler passage, with animals showing more intermediate patterns of structure, from *Acteon* to the more characteristic opisthobranchs than from *Acteon* to the more characteristic prosobranchs. As a matter of convenience, therefore, it seems appropriate to leave the animal where it has long been placed, as the most primitive type of living opisthobranch. This work on *Acteon*, and recent work on *Siphonaria* (Hubendick, 1947), on the ellobiid pulmonates (Morton, 1954), on *Onchidella* (Fretter, 1946), on *Omalogyra* and *Rissoella* (Fretter, 1948), on the pyramidellids (Fretter & Graham, 1949) and on other tectibranchs (Lloyd, 1952), demonstrate, however, with great emphasis, how close the relationship of all the three major groups of gastropods is at some central point such as this. It seems likely that further research will show that it is from some archaeogastropod nucleus, represented to-day by such molluscs as the trochids, that all these groups have arisen.

This work was carried out at the Plymouth Laboratory of the Marine Biological Association. Our thanks are due to Birkbeck College for a grant towards the cost of publication.

SUMMARY

Acteon tornatilis is an opisthobranch mollusc which burrows in sand, using foot, labial and cephalic tentacles in the process. The last also help to exclude sand from the mantle cavity, which is extended into a caecum coiling alongside the visceral hump, presumably used for respiration but also for excretion. The main pallial water current is an exhalant one on the right.

The mantle skirt carries, on the left, numerous repugnatorial glands with toxic secretions.

Labial glands lie at the mouth and the buccal cavity contains jaws and a reduced buccal mass and radula. Into it open salivary glands the structure of which is like that of the pyramidellids. The oesophagus shows traces neither of glands nor of torsion whereas the stomach, though simplified, has resemblances to that of a prosobranch.

The reproductive system is shown to be different from previous descriptions. Male and female ducts are separate from the lower end of the little hermaphrodite duct. The former passes to a prostate from which a vas deferens leads to a large uninvaginable penis; the latter has associated albumen and mucous glands and there is a receptaculum seminis to the duct of which a ventral channel leads from the female aperture in the mantle cavity.

Discussion of these aspects of the animal's structure confirms its position as the most primitive of the opisthobranchs.

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Abbreviations used in the Figures

A	anus
AG	tubules of albumen gland
AM	amoebocyte
C	columellar muscle
CC	cut end of pallial caecum
CM	pallial caecum
CR	ciliated ridge
CT	ctenidium
DD	opening of duct of digestive gland
DG	digestive gland
F	ventral channel leading to duct of receptaculum and to fertilization chamber
FA	lips of female aperture
FO	female opening
FT	foot
G	gonad
G_1, G_2, G_3	different types of pallial gland cells in *Patelloida*
GC	gland cell
GT	opening of glandular tubules into upper part of mucous gland
H	hypobranchial gland
HD	hermaphrodite duct
I	intestine
IPE	inner pallial epithelium
L	longitudinal strip of ciliated epithelium in hermaphrodite duct
LT	labial tentacle
LU	lumen of mucous gland
M	mouth
MA	opening of vas deferens at tip of penis
MC	mucous cell
MD	male deferent channel, a bifurcation of the little hermaphrodite duct
MF	muscle fibre
MG	tubules of mucous gland
NP	nerve plexus
O	oesophagus
OA	oesophageal aperture
OG	opening of repugnatorial gland
OPE	outer pallial epithelium
P	prostate gland
PE	penis
PG	pallial genital duct
PS	pedal surface
PT	posterior part of pallial oviduct formed of albumen gland and tubules of mucous gland
PV	pallial vein
R	receptaculum seminis
RD	duct of receptaculum seminis
RG	repugnatorial gland
S	exhalant siphon
SC	supporting cell
SS	style sac region of stomach
ST	stomach
T	cephalic tentacle
T_1, T_2,	major and minor typhlosoles
V	blood space of caecum
VC	ventral channel
VD	vas deferens
VP	vas deferens with prostatic gland cells projecting from the dorsal wall
VS	vesicula seminalis
VW	ventral wall of mucous gland overlying prostate

J. Mar. biol. Ass. U.K. (1954) 33, 587–588
Printed in Great Britain

THE POSITION OF *PONTOPHILUS ECHINULATUS* (M. SARS) IN THE CRANGONIDAE

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On 26 November 1953 the new research ship *Sarsia* on her first cruise collected a number of *Pontophilus echinulatus* (M. Sars) (with an Agassiz trawl, depth 100 fm., La Chapelle, Bay of Biscay). Mr G. R. Forster, of the Plymouth Laboratory, noted that these did not fit into the key given by Kemp (1910) in that they have in the male a distinct appendix interna at the base of the four last pairs of pleopods, while in the female this is absent, the inner ramus being usually undivided, although occasionally an indistinct division can be made out. Otherwise they agree with Kemp's diagnosis.

Kemp (1910) notes M. Sars 1861 as authority, but this is only a preliminary description. In Sars (1868), given in Kemp's (1910) bibliography but not under the species, it is seen that he describes both male and female exactly as in the specimens from *Sarsia*, the male having a distinct appendix interna on the last four pairs of pleopods and an appendix masculina also on the second pair, the female pleopods having very short inner rami, except in the first which is long, and there is no appendix interna. Sars's figures of these limbs are very good. Kemp (1911) joins *Philocheras* with *Pontophilus*, and in this he is now followed by most authors.

Kemp (1916) reviewed the pleopods in the genus *Pontophilus*, as far as he could with the material he had in India, and he proposed certain groups according to the presence or absence of the appendix interna. His material was deficient in males of some species, including *P. echinulatus* which he suggests would probably belong to group V in which the appendix interna is absent in both sexes.

This problem was referred to Dr I. Gordon, who very kindly examined some of the material in the British Museum and found that both male and female agreed with Sars's figures. She also found that most of Kemp's Irish Fisheries' material is immature, although one (Helga CXXI), presumably that at the foot of p. 145 in Kemp (1910), had an appendix interna on pleopods 2–5, probably not noted by Kemp. Thus the immaturity of these Irish specimens accounts for the fact that *P. echinulatus* (as *Philocheras*) is placed among those species which have no appendix interna (Kemp, 1910, pp. 135, 146).

As it is now certain that *P. echinulatus* has in the male a distinct appendix interna and none at all in the female, it follows that of the groups formed by

Kemp *P. echinulatus* should be placed in group III in which are included his four new Indian species *P. lowisi*, *P. pilorus*, *P. candidus* and *P. plebs*. It is interesting to find that Holthuis (1952) describes the pleopods of *P. prionolepis* n.sp. exactly as in Sars's description of *echinulatus*. He states that *P. prionolepis* is closely related to *P. lowisi* Kemp which is in group III, thus emphasizing the probable correct position of *P. echinulatus*. However, *P. sculptus* (Bill) seems very close to *echinulatus* except for the pleopods. Kemp (1911) revised his diagnosis of *P. sculptus*, and again in 1916, as it was found to have an appendix interna on the last four pairs of pleopods in the male and on some of them in the female. In Kemp's (1911) table, if we substitute the presence of an appendix interna instead of the absence in *P. echinulatus*, we see that *echinulatus* and *sculptus* agree in every other point. Kemp (1916) shows that the female *sculptus* has an appendix interna on the second and third pleopods and a rudimentary one on the fourth, thus differing from *echinulatus*, and therefore that species cannot be included in group II.

It follows from the above notes that *P. echinulatus* should take a different position and no longer be classified with *P. bispinosus* and *P. trispinosus*, if the appendix interna is as important in showing relationship as Kemp suggests. *P. echinulatus* does not fit into group II and must, if placed in any of the groups, be included in group III with Kemp's four Indian and Holthuis's African species, none of which appear to be so near it as do *P. sculptus*, *P. bispinosus* and *P. trispinosus*. Except for the appendix interna, *P. echinulatus* seems to agree very well with these species.

The best one can do at present seems to be to keep Kemp's groups I–V, emphasizing the fact that *P. echinulatus* seems to be nearest *P. sculptus*, *P. bispinosus* and *P. trispinosus*, although these are in different groups.

Group I (with appendix interna in both sexes).
Group II (with appendix interna in ♂ and partly in ♀): *P. sculptus*, etc.
Group III (with appendix interna in ♂ only): *P. echinulatus*, etc.
Groups IV and V (no appendix interna): *P. bispinosus*, *P. trispinosus*, etc.

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J. Mar. biol. Ass. U.K. (1954) 33, 589–612
Printed in Great Britain

N.I.R.D. Paper No. 1536

VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

II. STUDIES OF SEASONAL VARIATIONS IN SOME MARINE CRUSTACEA

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(Text-figs. 1–4)

Introduction

We have previously discussed the richness in vitamin A of the euphausiids, *Meganyctiphanes norvegica* (M. Sars) and *Thysanoessa raschii* (M. Sars), (Fisher, Kon & Thompson, 1952, 1953). We found that astaxanthin was the principal carotenoid pigment and that β-carotene was present in minute amounts, or not at all, in free-swimming animals of these two species, in *Meganyctiphanes norvegica* taken from the stomachs of arctic baleen whales (Kon & Thompson, 1949*a*) and in *Euphausia superba* Dana from the stomachs of antarctic baleen whales (Kon & Thompson, 1949*b*).

Our findings contradicted those of Wagner (1939), who had claimed to have found high concentrations of β-carotene in euphausiids from the stomachs of arctic whales, but made no mention of the typical crustacean pigment, astaxanthin.

Moore (1950), in discussing our work, stated that 'in view of Goodwin's observations on the variations in the relative concentrations of astaxanthin and β-carotene in locusts it might be unwise to discredit Wagner's claim to have isolated β-carotene from krill without fully exploring the effects of age and season'. During his study of locusts, Goodwin (1950) found that, in developing eggs, β-carotene was present initially but was gradually replaced from the 6th or 7th day of incubation by astaxanthin. As the hoppers approached maturity, however, the astaxanthin content remained steady whereas β-carotene increased, especially so in the adult phases.

The work described in this paper was designed among others to study whether similar variations in carotenoids occurred in some euphausiid and decapod Crustacea, and whether in this way Wagner's findings could be reconciled with ours.

Material and Methods of Collection

A series of collections of *Meganyctiphanes norvegica* and *Thysanoessa raschii* was taken from Loch Fyne at about monthly intervals for 18 months, and the analytical results have now provided information about possible seasonal and developmental variations in the concentrations of vitamin A and carotenoids. Similar studies were made on *Nephrops norvegicus* Leach and *Crangon allmani* Kinahan taken during the same period from the Clyde Sea area, on *C. vulgaris* L. from Plymouth and Conway and on the eyes of *Homarus vulgaris* M.-Edwards.

The euphausiids, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, were taken from lower Loch Fyne with a 1 m stramin net at depths of 80–90 fathoms (146–165 m) at approximate intervals of 1 month from February 1951 until July 1952. Before the animals were killed each monthly haul was separated into size-groups by length, measured from the tip of the rostrum to the base of the telson, at 2 mm intervals. Intermediate lengths were grouped with the next smaller size.

Collections of *Crangon allmani* and *Nephrops norvegicus* were also made during these visits to Loch Fyne, the former caught with the Agassiz trawl and the latter with the otter trawl. Groups of the brown shrimp, *Crangon vulgaris*, were sent to us at frequent intervals, at first from the Plymouth Laboratory and later from the Fisheries Experiment Station at Conway, during the period from August 1951 to February 1953. One group was collected at Burnham-on-Crouch in March 1952.

All these specimens were preserved as soon as possible after catching by boiling in sea-water, as described by Fisher *et al.* (1952). The euphausiids and *Crangon* spp. were separated into eyes and bodies before analysis and the specimens of *Nephrops* were dissected into several parts. We were also supplied with eyes of *Homarus vulgaris* taken from lobsters boiled for catering.

Analytical Methods

Fat was extracted, and vitamin A and carotenoids separated from it by saponification and chromatography on alumina columns, as outlined by Fisher *et al.* (1952). Total carotenoids were measured with the photoelectric spectrophotometer of Thompson (1949), and vitamin A was determined by measurement with this instrument of the intensity of the blue colour produced in the Carr-Price reaction.

Vitamin A ester and alcohol were separated by chromatography before saponification (see Fisher *et al.*, 1952) in the analyses of groups of *Thysanoessa raschii* collected on 5–6 September 1951, and of both this species and *Meganyctiphanes norvegica* collected on 17–18 October 1951, and in subsequent months. Similar treatment was given to some groups of the decapod Crustacea.

We have also analysed groups of eyes of *M. norvegica* for retinene by a method used successfully to separate this compound from cephalopod eyes, to be fully described in a later paper. No retinene was detected in the eyes of *Meganyctiphanes*.

RESULTS

Euphausiacea

The graphs in Figs. 1 and 2 show the vitamin A and astaxanthin concentrations in relation to the length of the specimens in each size-group of *M. norvegica* (Fig. 1) and *Thysanoessa raschii* (Fig. 2). The average weight per specimen of each size-group is also plotted. The detailed results of all the analyses on which these graphs are based have been recorded by Fisher (1953).

Vitamin A was found predominantly in the eyes of both species, varying from 66 to 100% of the total quantity in *Meganyctiphanes norvegica* and from 65 to 98% in *Thysanoessa raschii*, and amounting usually to over 90%. Astaxanthin or its esters were the only carotenoids in any of the size groups of either species. In *Meganyctiphanes* the eyes contained from 18 to 77% of this pigment, the average proportion being about 50% of the total in the whole animal; in *Thysanoessa*, from 33 to 94% of the total astaxanthin was in the eyes, the average value being about 65%.

As mentioned already, most of the specimens were subjected to the full analytical treatment of Fisher *et al.* (1952) and the relative proportions of vitamin A alcohol and ester were determined. In *T. raschii* collected in September the ratio of vitamin A ester to alcohol varied between 6:1 and 28:1 in the eyes of different size-groups and between 1:1 and 2:1 in the bodies; in October the eyes had from 6:1 to 11:1 and the bodies 1:1 to 3:1. In the eyes of *Meganyctiphanes norvegica* in October, the ratio varied between 2:1 and 16:1, being about 6:1 in most groups, whereas in the bodies it was 1:1 in all groups except one in which it was 3:1. These considerably higher ratios of vitamin A ester to vitamin A alcohol in the eyes than in the bodies observed during September and October were also found in all the remaining groups analysed until the investigation ended in July 1952.

The size-groups studied included the later furcilia stages through to the largest adult forms. In none of the groups of either species was β-carotene ever found in more than minute traces. There was certainly never enough β-carotene to measure, and the measurement would have been possible even if only 1 μg of β-carotene had been present in an extract from several thousand euphausiids. It is unlikely, therefore, that β-carotene is ever present in *M. norvegica* and *Thysanoessa raschii* in quantities that would justify Wagner's (1939) claim.

In many of the groups of *Meganyctiphanes norvegica* collected from January to July 1952 and in those of *Thysanoessa raschii* collected from March to July 1952 the sexes were analysed separately, but no consistent differences between them in their contents and concentrations of vitamin A and astaxanthin were found and indeed, on many occasions, the results for the two sexes were remarkably close. We have obtained similar results for *Meganyctiphanes norvegica* collected at Monaco (Fisher *et al.*, 1953).

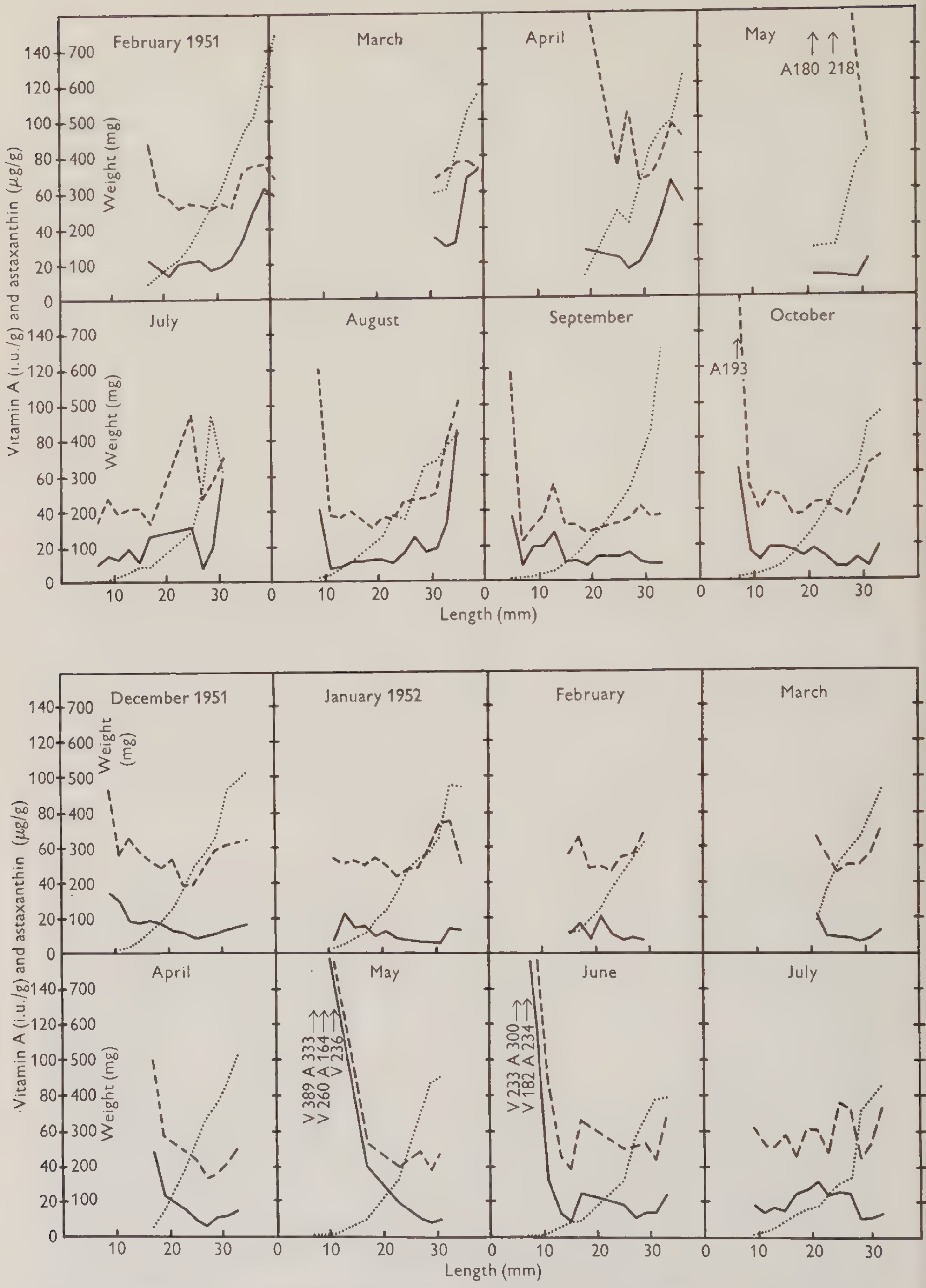

Fig. 1. Relationship between body-weight and concentrations of vitamin A and astaxanthin, and length, in *Meganyctiphanes norvegica*. V, vitamin A; A, astaxanthin; ——, vitamin A; - - - - -, astaxanthin; . . . . ., weight.

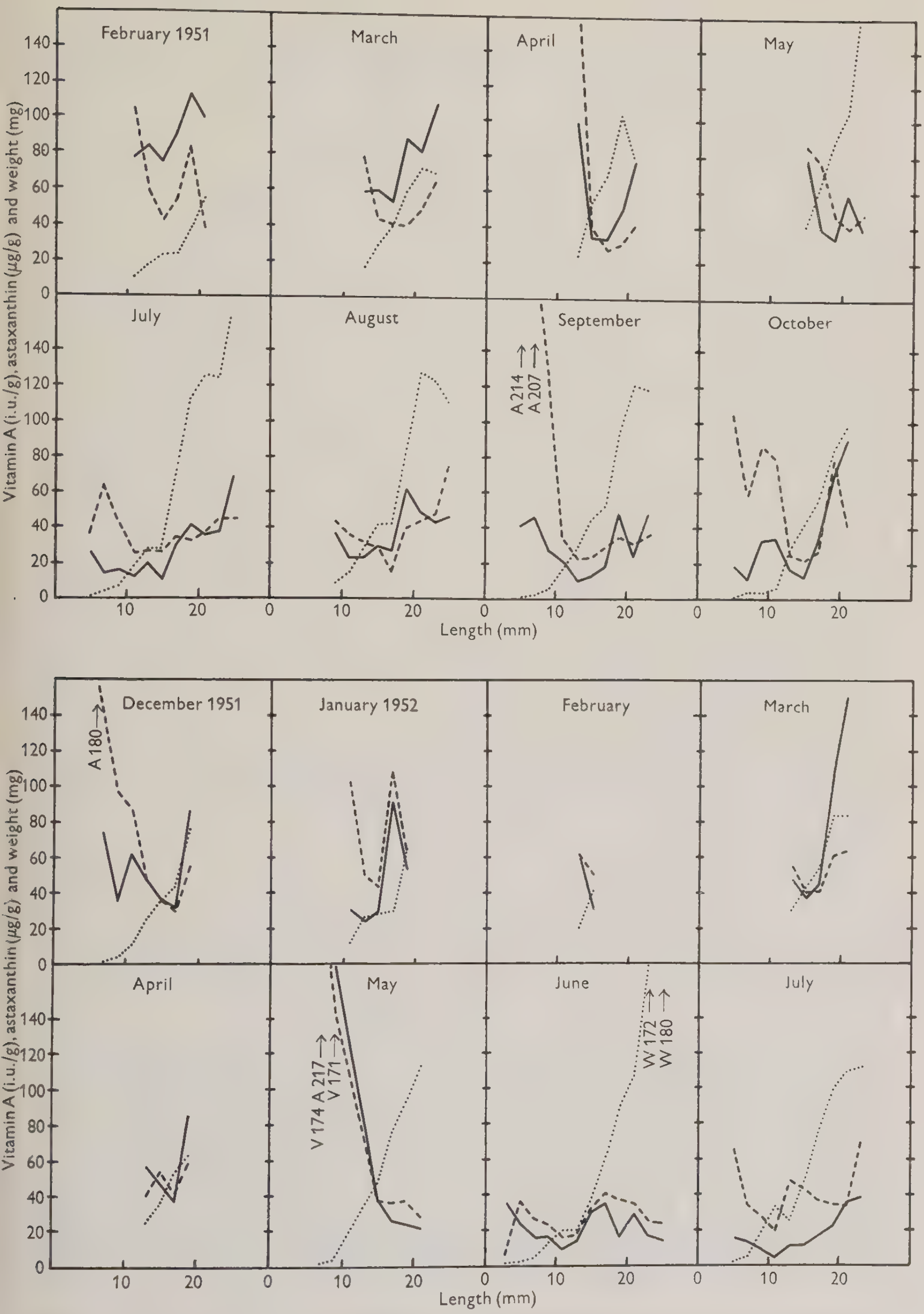

Fig. 2. Relationship between body-weight and concentrations of vitamin A and astaxanthin, and length, in *Thysanoessa raschii*. V, vitamin A; A, astaxanthin; W, weight; ——, vitamin A; - - - - -, astaxanthin; . . . . ., weight.

Many of the graphs in Fig. 1 show similarities in the fluctuations of the vitamin A and astaxanthin curves for *M. norvegica*. The concentrations reached their lowest levels in most of the monthly hauls in the size-groups between 20 and 30 mm. In the larvae concentrations were often very high, and in specimens over 30 mm long they tended to rise again, often quite steeply in some of the larger sizes. Unfortunately, after July 1951, only a very few specimens of *M. norvegica* longer than 30 mm were caught and no more good hauls of very large animals were obtained, such as were taken (Fisher *et al.*, 1952) during the autumn and winter of 1950–1, including February 1951, for which results appear in Fig. 1.

Although the vitamin A and astaxanthin curves showed parallel fluctuations, the relative values for astaxanthin became steadily higher during the period February–May 1951, whereas those for vitamin A remained at about the same level. From July until September 1951 the astaxanthin concentrations decreased, but those of vitamin A continued at their previous values. A second, but smaller rise in astaxanthin reserves was detectable during the next few months, reaching its peak in December 1951 or January 1952. The reserves declined slightly during the following period until the study ended in July 1952, and the increase in concentration observed during the spring of the previous year was not repeated. Vitamin A levels tended to be rather higher in June and July 1952 than at any time before.

The curves for concentrations of vitamin A and astaxanthin in *Thysanoessa raschii* (Fig. 2) fluctuated in much the same way as for *Meganyctiphanes norvegica*. Both substances tended to reach their lowest levels in animals between 15 and 19 mm long. The concentrations were highest in the larvae and in specimens over 19 mm in length. From February to July 1951 the concentrations of vitamin A and astaxanthin became lower and then remained at a fairly constant level until October, when they began to rise again to a maximum in January or February 1952. The decline observed in the previous spring was repeated in 1952, astaxanthin reaching its lowest level in June and increasing again in July, and vitamin A being at a minimum in July.

The graphs in Figs. 1 and 2 provide a useful picture based on analyses of the monthly collections, but, since the length-weight relationship changes seasonally as the animals feed and grow or fast and live on their reserves when food is scarce in the winter, seasonal variations can probably be studied better by following through the year one particular size-group. Length, rather than weight, is a better criterion of size because the girth may vary but the animals never become shorter and eventually, if not continuously, increase in length. In neither *M. norvegica* nor *Thysanoessa raschii* is the breeding period so restricted that all animals are simultaneously of the same size. Genetical and environmental variations would soon produce size differences even if this were so. *Meganyctiphanes norvegica* (Poulsen, 1926) and *Thysanoessa raschii* (Einarsson, 1945) usually breed from about March to June. The breeding periods appear to reach a peak, however, since, in our hauls of both species one size was almost always present in larger numbers than others. The 1 m stramin net was, therefore, regarded as a random sampler of the available population since it is likely that only very small larvae would escape from it. All the specimens in each haul were sorted into size-groups, as far as practicable, except when vast numbers of tiny larvae were taken. This method of selection did not take into account vertical or horizontal movements of populations within, and to and from, Loch Fyne, but euphausiids are caught there regularly and abundantly only in the deepest parts, and it is likely that a population would not move out entirely from an obviously favourable environment. With these reservations, we have considered the values for the size-group with the largest number of specimens in each monthly haul and regarded the series as representing the development of a homogeneous population. The fact that the length of the animals in the maximum-number groups either increased steadily or remained stationary for certain periods, but never regressed, during the year speaks for the soundness of this supposition. From the values so selected graphs were drawn showing the variations in vitamin A and astaxanthin content and concentration in *Meganyctiphanes norvegica* (Fig. 3) and *Thysanoessa raschii* (Fig. 4). Table I gives the average length of the specimens forming the selected group on each date. It was difficult to follow maximum-number groups from the larval stages since these, when present, were always in enormous numbers. The study covered the period from July 1951 to July 1952. In the first month, there were three size-groups of *Meganyctiphanes norvegica* with larger numbers of specimens than those next to them above or below, but in subsequent months only one group predominated in numbers. It appeared to correspond to the 15 mm size-group for July 1951 and this group was, therefore, used as starting-point in the graphs (Fig. 3). The group was probably hatched in the spring of 1951. Values for specimens of 13 mm length, which MacDonald (1927) regarded as first-year spawners, were the starting-points in the graphs for *Thysanoessa raschii* (Fig. 4). In all the graphs body-weight and fat concentration were also plotted.

The information contained in these graphs can now be considered in more detail under the headings of the various characteristics studied.

Size

In *Meganyctiphanes norvegica* (Fig. 3) the weight increased steadily from 47 mg in July to 246 mg in December, with a corresponding increase in length from 15 to 25 mm. From January to April 1952 both weight and length remained stationary, and then increased further to 434 mg and 33 mm by July. The two main periods of growth coincided with the spring and autumn diatom outbursts.

Thysanoessa raschii (Fig. 4) did not grow in the autumn to the same extent as *Meganyctiphanes norvegica*. The maximum-number group remained at 13 mm and 28 mg from July 1951 until October 1951 when the weight rose to 42 mg with no further change till March 1952. The length increased to 15 mm in December and remained so until March 1952. In April the length was 17 mm and the weight 54 mg; in May and

TABLE I. AVERAGE LENGTH IN MM OF SPECIMENS IN SELECTED GROUPS OF *MEGANYCTIPHANES NORVEGICA* AND *THYSANOESSA RASCHII* PRESENTED IN FIGS. 3 AND 4

Date	*M. norvegica*	*T. raschii*
5. vii. 51	15	13
16. viii. 51	17	13
6. ix. 51	21	13
18. x. 51	25	15
12. xii. 51	25	15
16. i. 52	25	15
13. ii. 52	25	15
13. iii. 52	25	15
24. iv. 52	27	17
22. v. 52	29	19
19. vi. 52	31*	19†
9. vii. 52	33‡	19§

* Largest group was of specimens 15 mm long, but the series considered here was 1 year older and, among these, animals 31 mm long formed the largest group.

† Largest group was of specimens 7 mm long, but the series considered here was 1 year older and, among these, animals 19 mm long formed the largest group.

‡ Corresponding maximum number for series was actually in 29 mm group, but specimens were both shorter and lighter than their predecessors in the series under consideration. The larger size-group completed the series more logically.

§ Largest group was of specimens 9 mm long, but series considered here was 1 year older and, among these, animals 19 mm long formed the largest group.

June the length was 19 mm and the weight 92 and 90 mg, and in July the length remained 19 mm and the weight had increased to 99 mg. As with *M. norvegica*, growth occurred during the spring diatom increase.

Fat

The fat concentration of the sample of *M. norvegica* (Fig. 3) collected in July 1951 (6·7 %, or 3 mg/specimen) was the highest for the whole year. There was a sharp drop in August to 2·4 % (2 mg/specimen) followed by a steady rise to 16 mg/specimen (6·4 %) in October and then a slight drop in December (5·6 % or 12 mg/specimen) and January (4·0 % or 10 mg/specimen), and an increase in February to 5·7 % (13 mg/specimen). In March the fat concentration dropped to a minimum for the year of 0·9 % (2 mg/specimen), increasing irregularly to 4·3 % (19 mg/specimen) by July 1952. Thus the general picture is one of increasing fat reserves during the autumn and spring feeding periods.

In *Thysanoessa raschii* (Fig. 4) the fat percentage increased from 7·0 % (2 mg/specimen) in July 1951 to 9·2 % (4 mg/specimen) in October 1951 and then steadily declined to a minimum of 1·3 % (0·5 mg/specimen) in February 1952. During the spring the fat reserves were replenished until they reached 5·2 % (5 mg/specimen) in July 1952. Autumn and spring feeding appear to have led to accumulation of fat which was used during the intermediate winter period when food was scarce.

The relatively sharp drop between February and March in *Meganyctiphanes norvegica* and between January and February in *Thysanoessa raschii* may have been associated with spawning.

Vitamin A

The content of vitamin A in *Meganyctiphanes norvegica* (Fig. 3) increased from 0·5 i.u./specimen in July 1951 to 1·9 i.u./specimen in September. Until April 1952 the

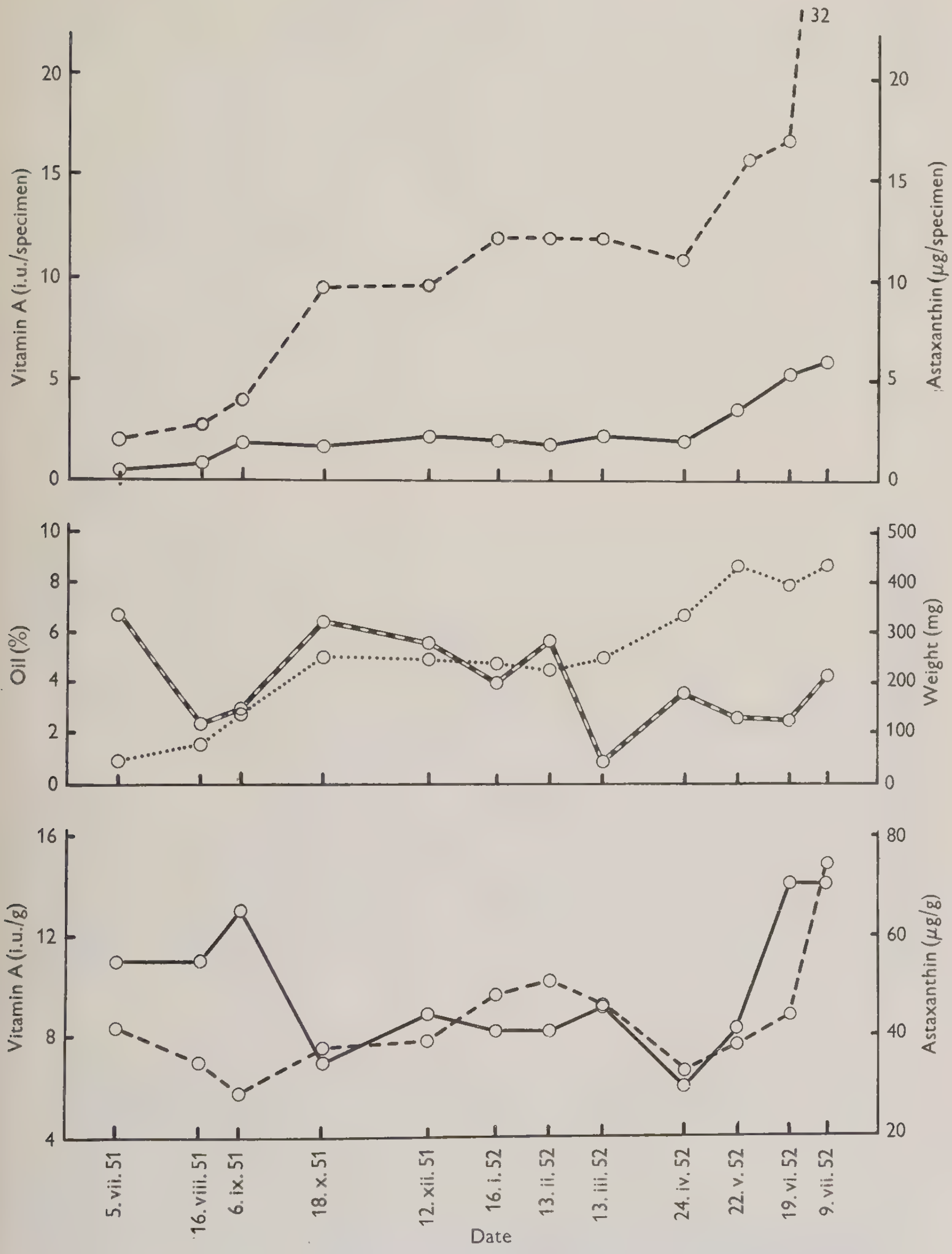

Fig. 3. Seasonal variations in the contents and concentrations of vitamin A and astaxanthin, oil percentage, and weight in selected groups of *Meganyctiphanes norvegica*. ——, vitamin A; - - - - -, astaxanthin; . . . . ., weight; ▬▭▬, oil.

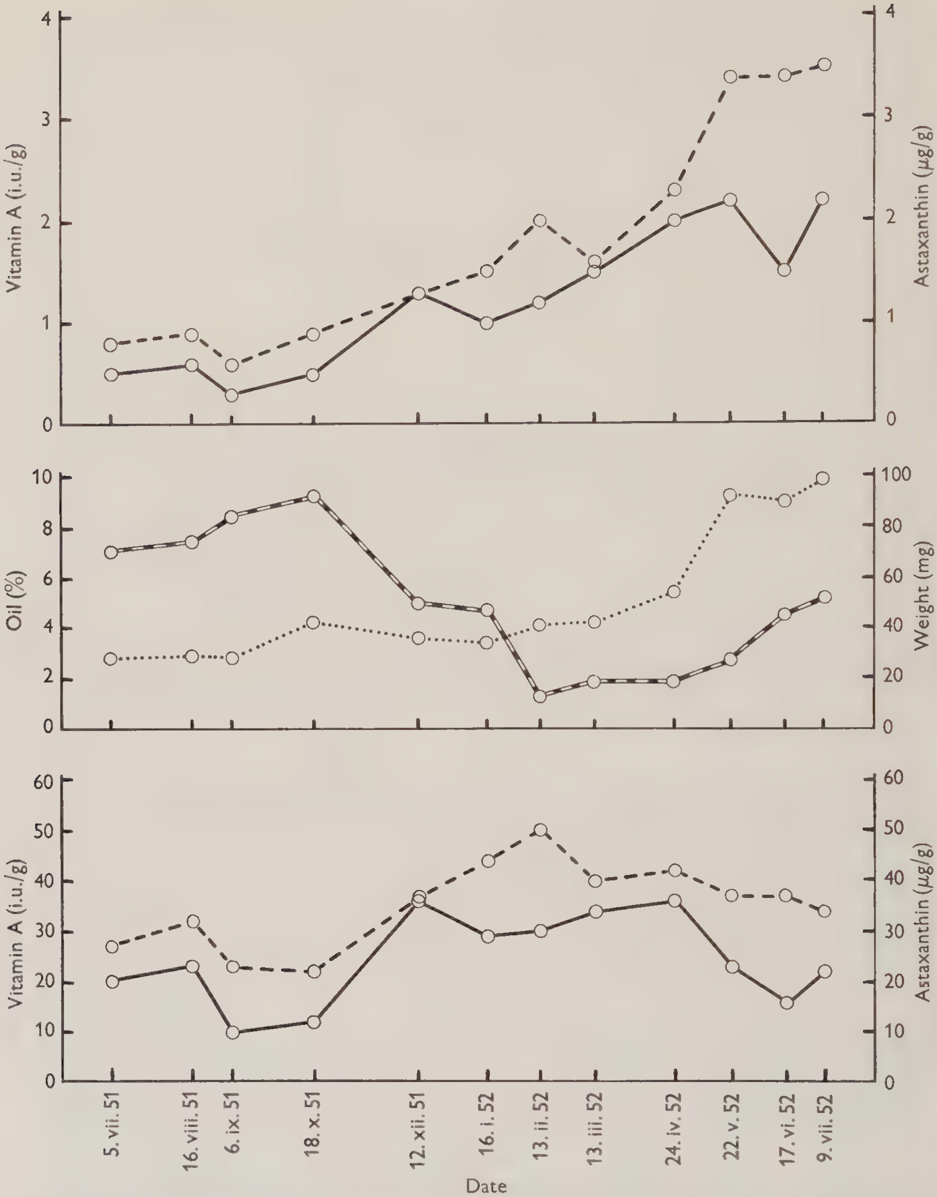

Fig. 4. Seasonal variations in the contents and concentrations of vitamin A and astaxanthin, oil percentage, and weight in selected groups of *Thysanoessa raschii*. ——, vitamin A; – – –, astaxanthin; ....., weight; ▬▭▬▭▬, oil.

value fluctuated around 2·0 i.u./specimen. It then climbed steadily to 6·0 i.u./specimen in July. The vitamin A concentration of *M. norvegica* (Fig. 3) increased from 11 i.u./g in July 1951 to 13 i.u./g in September, but decreased sharply in October to 7 i.u./g. During the following months it remained around 8–9 i.u./g, and then, in April, reached its lowest level, of 6 i.u./g; thereafter it climbed steeply to 14 i.u./g in June and July. The final concentration is rather low for animals of this size (see Fisher *et al.*, 1952).

The vitamin A content of *Thysanoessa raschii* (Fig. 4) was 0·5 i.u./specimen in July 1951 and remained at around this level until October. By December it had reached 1·3 i.u./specimen but increased no further until April, when it went up to 2·0 i.u. In May it was 2·2 i.u., regressed to 1·5 i.u. in June, and recovered to 2·2 i.u./specimen in July. The vitamin A concentration in the July 1951 sample of *T. raschii* (Fig. 4) was 20 i.u./g, and rose to 23 i.u./g in August. By September, it had fallen to 10 i.u./g and then increased, through 12 i.u./g in October to 36 i.u./g in December.

The concentration was somewhat lower in January and February but reached the December level again in March and April. Thereafter it declined to 23 i.u./g in May and to 16 i.u./g in June, climbing once more to 22 i.u./g in July 1952. As in *Meganyctiphanes norvegica* the concentrations of vitamin A in *Thysanoessa raschii* are also somewhat lower than found by us previously (Fisher *et al.*, 1952).

Astaxanthin

The astaxanthin content of *Meganyctiphanes norvegica* (Fig. 3) increased 16-fold from 2 μg/specimen in July 1951 to 32 μg/specimen in July 1952 compared with a 12-fold increase in vitamin A during the same period. This increase in astaxanthin content occurred in two periods, one during the autumn months, reaching 10 μg/specimen in December, and the second from 11 μg in April to 17 μg in June, with a final steep climb to 32 μg in July. The concentration of astaxanthin in *M. norvegica* (Fig. 3) decreased from 42 μg/g in July 1951 to 29 μg/g in September, when it rose once more, reaching its highest level in February of 51 μg/g. It then steadily declined until April to 33 μg/g. Thereafter it increased to 44 μg/g by June and to 74 μg/g in July.

In *Thysanoessa raschii* (Fig. 4) the astaxanthin content in July 1951 was 0·8 μg/specimen and remained at around this level until October. It increased steadily during the next few months and by February was 2·0 μg/specimen. After a slight drop in March, the increase continued to 3·4 μg/specimen in May when the content levelled off for the rest of the period until July. Astaxanthin concentrations in *T. raschii* (Fig. 4) fluctuated during the autumn months from 27 μg/g in July 1951 to 22 μg/g in October, and then steadily increased to attain their peak in February 1952 at 50 μg/g. In March 1952 the concentration fell to 40 μg/g and decreased slightly during the next few months to 34 μg/g when the study ended in July.

Caridea

Crangon allmani was collected from Loch Fyne less regularly than the euphausiids. The results are given in Table II. Vitamin A was present, always in the ester form, in all groups except that collected in September 1951 and, when found, was chiefly, and often exclusively, in the eyes, but in concentrations much lower than in the euphausiids. The carotenoids of *C. allmani* included astaxanthin or its esters, carotenes and xanthophylls.

C. vulgaris (Table III) had vitamin A in all groups, confined exclusively to the eyes. The ester predominated in all those groups in which the two forms were separated. The concentrations of vitamin A in this species were usually lower than those found in *C. allmani*, but β-carotene was found in a larger proportion of, though not in all, groups of *C. vulgaris*.

The principal carotenoid in both species of *Crangon* was astaxanthin and its concentration in the eyes, not given in Table III, was about 10 times that in the bodies, although the eyes contained only about 5% of the total carotenoids in the whole animal, the bulk of the pigments being xanthophylls.

There was no indication of any seasonal variation in either vitamin A or carotenoids in these two species of *Crangon*.

TABLE II. DISTRIBUTION OF OIL PERCENTAGE, VITAMIN A AND CAROTENOIDS PER SPECIMEN AND PER GRAM IN WHOLE ANIMAL (A) AND PAIRS OF EYES (E) OF *CRANGON ALLMANI* COLLECTED FROM LOCH FYNE

					Vitamin A		Carotenoids*	
Date	Tissue	No. of specimens	Wt. (mg)	Oil (%)	i.u./specimen	i.u./g.	μg/specimen	μg/g
6. ix. 51	A	17	403	2·1	0	0	1·2	3·0
	E	17	2	1·6	0	0	0·05	25
18. x. 51	A	11	260	4·2	0·28	1·1	1·1	4·2
	E	11	1	2·9	0·15	150	0·05	50
13. iii. 52	A	18	1109	1·8	0·15	0·1	1·8	1·6
	E	18	5	0·4	0·15	32	0·08	17
24. iv. 52	A	10	514	0·1	0·14	0·3	0·6	1·2
	E	10	3	5·5	0·14	50	0·4	132
22. v. 52	A	71	829	0·7	0·11	0·1	0·6	0·8
	E	71	4	11	0·11	27	—	—

* β-carotene absent.

Astacura

Nephrops norvegicus was collected in small groups from Loch Fyne during the visits made to collect euphausiids between July 1951 and July 1952. The animals were dissected and various organs were analysed in separate groups. The results are given in Table IV. From February 1952, the specimens were separated into eyes, gizzard, hepatopancreas and rest of body. As previous experience had shown that this last portion contained little or no vitamin A, expenditure of large volumes of solvents for the extraction of the large bulk of material in a number of bodies seemed unnecessary and to study any seasonal variation we confined our attention to the organs mentioned separately above.

In the group collected on 14 August 1951 the eye-stalks were separated from the eyes to determine whether the vitamin A in the eyes was associated with the sinus gland of the eye-stalk, but all the vitamin was in the eyes themselves. In contrast to the shrimps, the carotenoid pigment in the eyes was in concentrations of a similar order to those in the rest of the body and was exclusively astaxanthin. Carotenes and xanthophylls were present with astaxanthin in the other parts of the animals and small measurable amounts of β-carotene were found in some groups, usually in the hepatopancreas. There

Table III. Oil Percentage, Vitamin A per Specimen and per Gram, Carotenoids per Specimen, per Gram and per Gram Oil, and β-Carotene per Gram Oil in Groups of *Crangon vulgaris* from several Localities

Specimens analysed as bodies and eyes, but all Vitamin A was in the eyes and only total figures are given here

Date	Locality	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Total carotenoids			β-carotene, μg/g oil
					i.u./specimen	i.u./g	μg/specimen	μg/g	μg/g oil	
21. viii. 51	Plymouth	93	680	1·1	0·05	0·08	5·2	7·6	691	41
19. ix. 51	Plymouth	52	1055	0·8	0·06	0·06	2·3	2·2	276	28
27. xi. 51	Colwyn Bay	161	1176	1·2	0·08	0·07	4·9	4·1	357	17
12. xii. 51	Abergele	164	1239	1·0	0·06	0·05	4·2	3·4	340	59
2. i. 52	Conway	148	1360	1·4	0·06	0·05	6·2	4·5	326	33
16. i. 52	Conway	62	873	1·6	0·10	0·11	2·9	3·3	212	—
16. i. 52	Conway	92	568	1·4	0·06	0·11	1·8	3·1	224	—
16. i. 52	Conway	72	292	1·8	0·06	0·19	1·2	4·1	225	—
26. i. 52	Colwyn Bay	79 (*a*)	1819	1·9	0·07	0·04	8·1	4·5	224	11
26. i. 52	Colwyn Bay	91 (*b*)	647	1·2	0·08	0·12	2·5	3·8	325	0
28. i. 52	Holyhead	58 (*a*)	1499	1·3	0·08	0·05	6·7	4·5	338	22
28. i. 52	Holyhead	114 (*b*)	798	1·2	0·08	0·10	2·4	3·0	250	4·6
11. ii. 52	Colwyn Bay	40	2204	1·8	0·09	0·04	7·0	3·2	175	9·0
11. ii. 52	Colwyn Bay	80	1032	1·8	0·09	0·08	3·8	3·7	208	7·1
11. ii. 52	Colwyn Bay	70	549	1·5	0·04	0·07	1·5	2·7	187	1·9
25. ii. 52	Pendwffyn	18 (*a*)	1223	2·0	0·09	0·07	3·8	3·1	157	—
25. ii. 52	Pendwffyn	57 (*b*)	600	1·5	0·06	0·10	2·0	3·3	215	—
26. ii. 52	Colwyn Bay	37 (*a*)	1706	1·7	0·09	0·05	8·0	4·5	271	—
26. ii. 52	Colwyn Bay	53 (*b*)	707	1·7	0·09	0·13	2·5	3·6	211	—
25. iii. 52	Burnham-on-Crouch	92	1125	1·2	0·06	0·05	5·7	5·8	497	1·9
18. iv. 52	Conway	130	1229	2·2	0·13	0·11	6·1	5·0	226	1·0
22. v. 52	Colwyn Bay	165	1021	1·4	0·09	0·09	4·0	3·9	277	0·7
5. viii. 52	Llanfairfechan	129	1352	1·4	0·04	0·03	7·1	5·3	370	0·7
21. viii. 52	Colwyn Bay	107	1003	1·1	0·05	0·05	4·5	4·5	420	0·3
4. x. 52	Pendwffyn	190	686	0·6	0·04	0·05	2·3	3·4	544	25
23. x. 52	Pendwffyn	119	1088	0·9	0·06	0·06	4·3	3·9	443	15
17. xi. 52	Rhyl	101	1406	1·8	0·07	0·05	5·3	3·8	211	0
18. xii. 52	Pendwffyn	148	1053	0·8	0·07	0·06	7·1	6·7	413	0
9. ii. 53	Conway	192	1105	1·8	0·04	0·04	5·5	5·5	272	—

(*a*) ‘Berried’ ♀. (*b*) Mixed ♂ and ♀ without eggs.

TABLE IV. DISTRIBUTION OF OIL PERCENTAGE, VITAMIN A PER SPECIMEN AND PER GRAM, CAROTENOIDS PER SPECIMEN, PER GRAM AND PER GRAM OIL, AND β-CAROTENE PER GRAM OIL IN *NEPHROPS NORVEGICUS* FROM THE CLYDE SEA AREA

For eyes and eye-stalks all values are per pair

Date	Organ	No. of specimens	Av. wt. (g)	Oil (%)	Vitamin A		Total carotenoids			β-carotene, µg/g oil
					i.u./specimen	i.u./g	µg/specimen	µg/g	µg/g oil	
3. vii. 51	Whole animal	5	76	1·5	3·0	0·04	673	8·9	593	7·8
	Eyes	5	0·4	1·3	1·2	2·8	4·1	9·5	715	0
14. viii. 51	Whole animal	6	121	0·9	3·4	0·03	965	8·0	889	18
	Eyes	6	0·5	1·5	2·2	4·7	5·7	12	793	0
	Eye-stalks	6	0·07	8·5	0	0	0·8	12	137	0
4. ix. 51	Whole animal	6	132	2·0	12	0·09	741	5·6	280	4·7
	Eyes	6	0·6	0·9	2·4	4·0	5·6	9·2	1022	0
	Hepatopancreas	6	7·8	29	9·3	1·2	61	7·9	27	5·2
	Alimentary canal	6	2·5	0·7	0	0	23	9·4	1343	0
16. x. 51	Whole animal	6	108	1·3	3·2	0·03	792	7·3	551	12
	Eyes	6	0·6	0·4	2·0	3·5	2·4	4·2	1141	0
	Hepatopancreas	6	6·0	20	1·2	0·2	36	6·0	30	12
11. xii. 51	Whole animal	6	203	1·3	6·1	0·03	1218	6·0	453	8·8
	Eyes	6	0·7	0·3	4·1	5·7	5·5	7·6	2523	0
	Gizzard	6	2·6	0·3	0	0	51	19	5972	0
	Hepatopancreas	6	10	20	0·9	0·1	62	6·1	31	11
15. i. 52	Whole animal	6	211	0·6	4·6	0·02	916	4·3	767	13
	Eyes	6	0·8	0·2	3·9	5·1	5·8	7·5	3165	0
	Gizzard	6	2·9	0·2	0	0	17	6·1	2568	0
	Hepatopancreas	6	8·1	10	0·7	0·1	22	2·7	27	18
12. ii. 52	Whole animal	6	176	—	1·8	0·01	—	—	—	—
	Eyes	6	0·7	0·9	1·8	2·5	4·4	6·1	686	0
	Gizzard	6	3·8	0·5	0	0	4·7	1·2	239	0
	Hepatopancreas	6	8·9	13	0	0	26	2·9	22	4·4
11. iii. 52	Whole animal	3	144	—	2·6	0·02	—	—	—	—
	Eyes	3	0·6	0·8	2·6	4·1	6·7	11	1329	0
	Gizzard	3	2·2	0·8	0	0	11	5·0	632	0
	Hepatopancreas	3	7·1	19	0	0	69	9·6	52	0·6
24. iv. 52	Whole animal	4	200	—	2·1	0·01	—	—	—	—
	Eyes	4	0·8	0·2	2·1	2·6	2·8	3·4	2298	0
	Gizzard	4	3·1	0·3	0	0	19	6·0	1742	0
	Hepatopancreas	4	9·5	9·8	0	0	50	5·8	59	19
23. v. 52	Whole animal	9	143	—	4·7	0·03	—	—	—	—
	Eyes	9	0·6	0·2	4·0	6·4	5·7	9·1	5120	0
	Gizzard	9	2·6	0·3	0	0	13	5·0	1527	0
	Hepatopancreas	9	7·5	8·3	0·8	0·1	46	6·1	74	16
17. vi. 52	Whole animal	11	165	—	1·4	0·01	—	—	—	—
	Eyes	11	0·7	0·2	1·4	1·9	1·9	2·6	1340	0
	Gizzard	11	3·3	0·3	0	0	18	5·4	1736	0
	Hepatopancreas	11	8·1	4·6	0	0	22	2·8	59	17
9. vii. 52	Whole animal	4	169	—	4·8	0·03	—	—	—	—
	Eyes	4	0·6	0·7	4·8	7·7	6·7	11	1611	0
	Gizzard	4	2·7	0·5	0	0	20	7·5	1520	0
	Hepatopancreas	4	8·1	6·7	0	0	28	3·5	53	20

was no consistent seasonal variation in the concentrations of vitamin A, carotenoids or fat in any of the organs investigated. The hepatopancreas was much richer in fat than the other parts of the animal.

Of *Homarus vulgaris*, only groups of eyes were analysed and the results are shown in Table V. Concentrations of both vitamin A and carotenoids, the latter predominantly astaxanthin with a little xanthophyll, were fairly high during the early months of 1951, but by July the vitamin A level had dropped to a value from which it deviated little during the remaining period of investigation up to April 1952. The content and concentration of carotenoids was fairly uniform throughout 1951, but in January 1952 it dropped to about half the previous level and persisted so in the following months until April.

Table V. Oil Percentage and Vitamin A and Carotenoids per Pair and per Gram in the Eyes of *Homarus vulgaris* of unknown Origin

Date	No. of eyes	Wt. (mg/pair)	Oil (%)	Vitamin A		Carotenoids	
				i.u./pair	i.u./g	μg/pair	μg/g
15. i. 51	57	686	0·4	10	15	40	54
19. ii. 51	50	743	0·7	9·9	13	44	59
28. iii. 51	52	646	0·8	12	17	43	66
16. iv. 51	50	739	0·7	15	20	44	59
vi. 51	56	713	0·7	6·5	9·1	38	53
vii. 51	16	599	1·6	3·7	6·2	51	85
10. ix. 51	55	710	0·5	4·2	6·0	28	39
5. xi. 51	58	735	0·6	2·8	3·8	24	33
5. xii. 51	50	488	0·9	3·0	6·1	30	62
19. xii. 51	55	534	1·4	3·0	5·4	32	58
28. i. 52	51	655	1·3	3·5	5·3	12	19
ii. 52	50	656	0·6	4·4	6·7	16	25
iii. 52	50	688	1·0	4·1	5·9	17	25
7. iv. 52	50	804	0·4	4·9	6·1	17	22

Discussion

The foregoing observations indicate that, whereas the fat, vitamin A and astaxanthin reserves of the euphausiids varied markedly with the season, no such changes occurred in the shrimps, *Crangon allmani* and *C. vulgaris*, or in *Nephrops norvegicus*. Vitamin A and carotenoids in the eyes of *Homarus vulgaris* varied to some extent with the season. These eyes weighed between 0·5 and 0·8 g per pair and were all preserved, stored and analysed in the same way, but the origin of the lobsters was unknown and may have varied through the year. This might account for the absence in 1952 of the trend observed in the previous year, when vitamin A and carotenoids increased during the spring to a maximum in April.

An interesting comparison between the two species of *Crangon* is noteworthy. The concentration of vitamin A in the eyes of *C. allmani* was higher than in those of *C. vulgaris*. The difference may be associated with the relative visual requirements of the two animals, as *C. allmani* lives at much greater depths and, therefore, in much darker surroundings than *C. vulgaris*, although both are benthic animals in which vision may be comparatively unimportant. On the other hand, it is possible that *C. allmani* from Loch Fyne also inhabited by large populations of vitamin A-rich euphausiids may have, by feeding on their living or dead bodies, accumulated its reserves at a faster rate than *C. vulgaris* from an environment where its food contains much less vitamin A. Admittedly, though *C. allmani* is carnivorous, we do not know whether it in fact preys on euphausiids. Another possibility is that both obtain their vitamin A by the conversion of a precursor such as β-carotene and that the process operates more efficiently in *C. allmani*, which usually had no β-carotene, than in *C. vulgaris* which contained it in most parts of the body.

We have established for a considerable range of sizes that the presence of the bulk of the vitamin A and of at least half the total astaxanthin in the eyes of both *Meganyctiphanes norvegica* and *Thysanoessa raschii* is a characteristic independent of the season. We have confirmed our earlier findings (Kon & Thompson, 1949*a*; Fisher *et al.*, 1952) that euphausiid vitamin A occurs mainly in the ester form, but we now know that this form predominates only in the eyes. Ester and alcohol were usually present in about equal amounts in the small quantity of vitamin A found in the bodies.

The ester is the form of vitamin A stored in the liver by vertebrates and the alcohol is the active form passed to the rest of the body in the blood stream. Thus most of the vitamin A in the euphausiid eye is in the storage form with only a small amount in the active state.

In the studies reported here and previously (Fisher *et al.*, 1952, 1953), we found the concentration of vitamin A in the eyes of *Meganyctiphanes norvegica* to be within the range of 2000–20,000 i.u./g dry weight and even if 95% of this is ester, the concentration of vitamin A alcohol would be between 100 and 1000 i.u./g. Such quantities would certainly be adequate for visual purposes, since, as we have previously pointed out (Fisher *et al.*, 1952), Wald (1935) found that in mammalian retinas the concentration of vitamin A adequate for vision is about 70 i.u./g dry weight and in frog retinas about 1200 i.u./g, and Morton & Rosen (1949) recorded a maximum value for whole eyes of frogs equivalent to 300 i.u./g dry weight.

Retinene (vitamin A aldehyde) which participates in the visual cycle of higher animals (Wald, 1945) is absent. Thus vitamin A in the eyes of euphausiids is apparently in excess of that required for vision. What, therefore, is its function, if any? In Crustacea, the hepatopancreas is a digestive organ and not a storage organ like the vertebrate liver. Both vitamin A ester and astaxanthin are found in the euphausiid eye in much higher concentrations than in the eyes or bodies of other animals and there is no obvious function for these large quantities. It may be that they are no more than metabolic end-products accumulating there during the life of the euphausiids, like vitamin A and carotenoids in, for example, fish livers (Macpherson, 1933).

Figs. 3 and 4 show that *M. norvegica* and *Thysanoessa raschii* accumulated vitamin A and astaxanthin mainly during the autumn and spring when diatom outbursts stimulated feeding. In *Meganyctiphanes norvegica* the concentrations of vitamin A reached maxima in June–July and in September, falling during the winter when growth continued but the vitamin A was not being accumulated. The astaxanthin concentration remained fairly high all winter in *M. norvegica*, dropping with that of vitamin A in April, a month after the fat concentration reached its minimum. Thus there was a time-lag between the resumption of growth at the faster spring rate, which probably began between February and March in *M. norvegica* and between January and February in *Thysanoessa raschii*, and an increase in the fat reserves, which began a month later, and another month passed before the contents of vitamin A and astaxanthin began to increase. Spawning may have occurred at around this time and depleted the stocks of fat, vitamin A and astaxanthin.

Meganyctiphanes began accumulating vitamin A and astaxanthin again in April (Fig. 3), whereas in *Thysanoessa* the concentration of vitamin A did not begin to rise until June or July (Fig. 4) and, even then, that of astaxanthin was still falling.

The difference between the two species may possibly be explained in terms of their food. *Meganyctiphanes norvegica* of the size considered here would be carnivorous (Macdonald, 1927), whereas the smaller *Thysanoessa raschii* would probably be mainly herbivorous, although less is known about the feeding of this species. Gillam, el Ridi & Wimpenny (1939) found that both phytoplankton and zooplankton reached their highest growth in May and August but the plant concentrations were much more transitory than those of animals. Zooplankton densities remained fairly high for 2 or 3 months after the peaks, whereas those of the phytoplankton soon became much lower. *Meganyctiphanes norvegica* might thus have a continued supply of adequate animal food but *Thysanoessa raschii* would have to subsist on rapidly diminishing stocks of plants.

Further evidence of a difference in feeding habits between *Meganyctiphanes norvegica* and *Thysanoessa raschii* is that the former (Fig. 1) accumulated astaxanthin much more rapidly than vitamin A, during spring 1951, whereas in *T. raschii* (Fig. 2) the two substances increased at a more closely similar rate. A greater proportion of zooplankton, composed predominantly of copepods and thus rich in astaxanthin, in the diet of *Meganyctiphanes norvegica* would account for its increased stores of this carotenoid. Another explanation may be found in the larger pigmented areas of the body in *M. norvegica* than in *Thysanoessa raschii*. Our analytical results have shown that a much higher percentage of the total astaxanthin is in the eyes, and, therefore, less is in the bodies, of *T. raschii*, than in those of *Meganyctiphanes norvegica*, which, therefore, requires more astaxanthin for its pigmentation.

In neither species of euphausiid was the accumulation of vitamin A and astaxanthin as marked in the spring of 1952 as in the previous year. The lower concentrations of these substances found during the winter months of 1951–52 (Figs. 1, 2) may, in some way, have been associated with the absence from our hauls of very large specimens of *M. norvegica* and the reduction in the numbers of both this species and *Thysanoessa raschii* during these months and the following spring. It is interesting that, in February 1952, fishermen at Monaco were complaining that *Meganyctiphanes norvegica* appearing in the port were smaller than usual at that time of year.

Examination of Fig. 4 shows that in *Thysanoessa raschii* there was a marked inverse relationship between the concentrations of fat and vitamin A through the year, and a similar relationship was also detectable in *Meganyctiphanes norvegica* (Fig. 3). The feeding habits of the animals probably account for the phenomenon. Fig. 4 indicates that *Thysanoessa raschii* lived mainly on its reserves during the period from October to March. Body-weight remained fairly constant but the stores of fat were reduced considerably. Continued accumulation of vitamin A and astaxanthin either from food eaten or by conversion from a precursor within the body resulted in increased concentrations of these substances. With the resumption of large-scale feeding in the spring, growth was more rapid and fat was again stored with the result that the concentrations of vitamin A and astaxanthin fell as these accumulated less rapidly than fat owing to the time-lag discussed above. Cause and effect are less easily elucidated in *Meganyctiphanes norvegica*, but there is no doubt that the inverse relationship is also found in this species. Gillam *et al.* (1939) noted a similar inverse relationship in their study between the vitamin A concentration and fat-free solids of gross plankton over the period of a year, apart from the spring increase when vitamin A increased as well. They suggested that the relationship was indicative of the type of behaviour expected from an autocatalyst of growth. It may, however, merely be a measure of the proportions in the total plankton of various planktonic organisms. At the peak periods for fat-free solids, the plankton would be composed mainly of plants and smaller Crustacea, such as copepods which, we know, lack vitamin A. This mixed population would be grazed down by larger animals, especially fish larvae and euphausiids, which form or accumulate vitamin A and concentrate it in their bodies. The population density of these organisms would be relatively much smaller than that of copepods and diatoms, so that the concentration of fat-free solids would be reduced, especially as most of the diatoms die and sink to the bottom soon after their outbursts, thereby further reducing the solid material present.

All our evidence points to the richness in vitamin A of euphausiids in comparison with other Crustacea. In Loch Fyne, *Meganyctiphanes* and *Thysanoessa* were taken in the same hauls as *Calanus finmarchicus* (Gunnerus) and *Euchaeta norvegica* Boeck and very often, near the bottom, with *Crangon allmani* and *Pandalus bonnieri* Caullery. Neither the copepods nor the decapods contained vitamin A in amounts approaching those in the euphausiids. The question arises what source of the vitamin or its precursors is available to these animals and closed to their neighbours. We believe that our study may have shed a little light on the problem. An examination of the monthly graphs for *Meganyctiphanes* in Fig. 1 shows that the highest concentrations of vitamin A and astaxanthin were found in the smallest and largest specimens, although the content of both vitamin A and astaxanthin increased throughout life. It seems very likely that in the former the vitamin and the pigment are passed on to the larva from the egg. But why do the concentrations increase steeply in animals over 30 mm long? This size is not associated with the achievement of maturity, since adult animals are found upwards of 20 mm long. The high concentrations were observed in large specimens at all times of year and so were not connected specifically with the breeding period.

Thysanoessa raschii showed certain of the trends noted in *Meganyctiphanes norvegica*. The increase in vitamin A and astaxanthin concentrations usually appeared in animals between 15 and 20 mm long. Again these are not related to the attainment of sexual maturity which occurs in this species at about 13 mm length (Macdonald, 1928).

We have examined the gut contents of *Meganyctiphanes norvegica* of different sizes. Smaller animals (15–25 mm long) eat much detritus of vegetable origin, together with diatoms, of which fragments were found in the gut. In the larger specimens (over 30 mm long), detritus was also present, but accompanied there by crustacean fragments, mainly of copepod origin and often identifiable as parts of *Calanus finmarchicus* and *Euchaeta norvegica*. Macdonald (1927) made a more systematic investigation of the food of *Meganyctiphanes norvegica* and reached the following conclusions:

'(1) Organic detritus is eaten most abundantly during the first months of the year; (2) *Meganyctiphanes*, ranging from 21–29 mm, feed more extensively on vegetable detritus than do larger or smaller specimens (it should be noted that this size was by far the most abundant in the Clyde Sea area); (3) Copepods are eaten most extensively by the larger specimens viz. 31–39 mm and (4) the smaller specimens, 13–19 mm, feed most extensively on diatoms and "wet dust"'.

'Wet dust' was also called, by Macdonald, flocculent detritus and consisted of a mass of greenish brown unidentifiable particles, including shells of diatoms and peridinians, spores of algae and possibly argillaceous particles. The species of diatoms he found most abundantly in *M. norvegica* were *Paralia sulcata* (Heiberg) Cleve, *Thalassiosira nordenskïoldi* Cleve, *T. gravida* Cleve and *Coscinodiscus* spp. Other diatoms occasionally eaten in quantities included *Nitzschia* and *Skeletonema*. We have analysed pure cultures of several diatoms, including *Thalassiosira gravida* Cleve, *Coscinodiscus concinnus* W. Sm., *Phaeodactylum tricornutum* Bohlin, and *Skeletonema costatum* (Grev.) Cleve and the dinoflagellate, *Peridinium trochoideum* (Stein) Lemm., and found no vitamin A in any of these organisms.

Little is known about the feeding of *Thysanoessa raschii*. Our own experience has been to find the gut empty except for occasional detritus or other unidentifiable material. It is difficult to postulate a change of diet, as in *Meganyctiphanes norvegica*, in animals so much smaller when the concentrations of vitamin A and astaxanthin begin to rise, unless the larger prey on larval copepods. We have never found, nor did Macdonald (1927), parts of euphausiids in the guts of others, so that it is very unlikely that larger euphausiids obtain their vitamin A preformed by eating smaller ones.

The change-over from mainly vegetable to mainly animal food, observed by both Macdonald and ourselves in specimens of *M. norvegica* over 30 mm long, might well be the cause of their increased rates of storage of vitamin A and astaxanthin, resulting from differences in the carotenoid constituents in the diet. The increased uptake and storage of astaxanthin would be thus explained because this pigment is the principal carotenoid in copepods. Vitamin A and astaxanthin are both absent, however, from diatoms and there is no vitamin A in *Calanus finmarchicus* (Euler, Hellström & Klussmann, 1934; Lederer, 1938; Fisher *et al.*, 1952), and only rarely in *Euchaeta norvegica* (Fisher *et al.*, 1952). Direct uptake of both substances is thus unlikely to account for their presence in the young herbivorous stages of *Meganyctiphanes*, and that of vitamin A for its presence in larger predominantly carnivorous animals. We must, therefore, look for precursors that these euphausiids can convert to astaxanthin and vitamin A. The plant food of the herbivores contains β-carotene, a known provitamin A and possibly also a precursor for astaxanthin. Neither β-carotene nor any other known provitamin A is found in more than trace amounts in the copepods and yet the larger stages of *M. norvegica* feeding on them appear capable of storing vitamin A much more rapidly than those living on plant material. They must, therefore, utilize as precursors either known carotenoids or other substances, normally not regarded as vitamin A precursors.

Let us consider the carotenoids present in the plankton organisms involved in these food-chains. The principal carotenoids in diatoms are β-carotene and xanthophylls, including certain pigments peculiar to these plants, such as diatoxanthin (Strain, Manning & Hardin, 1944). This carotenoid has an absorption spectrum very similar to that of zeaxanthin which, according to Goodwin (1952), is probably 3:3′-dihydroxy-β-carotene. Goodwin believes that diatoxanthin may be a *cis*-isomer of zeaxanthin and so possess the same structural formula. Astaxanthin is 3:3′-dihydroxy-4:4′-diketo-β-carotene (Kuhn & Sörensen, 1938), so that zeaxanthin, and, therefore, probably diatoxanthin, are structurally intermediate between β-carotene and astaxanthin. The oxidation of β-carotene to astaxanthin, if it occurs at all, may thus go as far as diatoxanthin in the diatoms and be completed in the zooplankton, giving rise to astaxanthin and vitamin A in euphausiids and to astaxanthin only in copepods. Zeaxanthin itself is inactive as a provitamin A for pigs (Braude *et al.*, 1941), but invertebrate carotenoid metabolism may be different, and it is interesting to note that recently Lenel (1953) found in the organs of the shore-crab, *Carcinus maenas* (Pennant), not only β-carotene and astaxanthin or their stereoisomers but also more or less oxidized pigments intermediate between these two. It is generally believed that Crustacea are unable to synthesize astaxanthin *de novo* (Sörensen, 1936; Fox, 1947). Fox suggested that pigments similar to astaxanthin may be formed in coelenterates, sponges, molluscs, echinoderms and other invertebrates, and that the common occurrence of acidic or acidogenic carotenoids among many invertebrate animals indicates a unique ability to effect a partial oxidation of commoner polyene molecules without actually splitting them. However, the only carotenoid molecule available in any quantity in its copepod diet to *Meganyctiphanes norvegica* is astaxanthin. If *Meganyctiphanes* converts this pigment to vitamin A, a reduction is involved but, as Fox (1953) points out, the general tendency in the animal body is towards the oxidation of carotenoids rather than to their reduction. The presence of oxygen in the β-ionone rings of carotenoids has always been considered by biochemists as a bar to their conversion to vitamin A, although Goodwin (1951) has suggested that this criterion may have to be modified since the work of Karrer, Jucker, Rutschmann & Steinlin (1945) on the production of carotenoid epoxides indicates that 5:6 epoxides, e.g. 5:6–5′:6′-diepoxy-β-carotene, are vitamin A precursors. As Goodwin pointed out, the formation of vitamin A may occur after initial conversion of the epoxides to β-carotene, in other words, after their reduction.

Our evidence certainly suggests that euphausiids utilize a vitamin A precursor not available to other Crustacea living around them, and it is difficult to ignore the coincidence between the appearance of astaxanthin in their diet and the appreciable increase in their vitamin A reserves.

The suggestion that astaxanthin may be a precursor for vitamin A is not entirely new; Drummond & MacWalter (1935) thought that astacin from krill might be converted to vitamin A by the whale. We now know (Kon *et al.*, 1949*a*, *b*) that there is no need for such a hypothesis since the vitamin is already present preformed in krill; Morton (1940) believed it possible that certain carnivorous animals, such as fishes, may be able to use astaxanthin from Crustacea in their food as a basis for conversion to vitamin A; and more recently Collins, Love & Morton (1953) have suggested astaxanthin as a possible precursor for vitamin A_2. In dealing with possible sources of vitamin A for euphausiids a further factor must be considered. Detritus is eaten by euphausiids of all sizes and is, therefore, unlikely to account for increased accumulation of vitamin A in larger euphausiids. Nevertheless, the importance of detritus as a source of carotenoids must not be overlooked (see Fox, 1950). Fox, Isaacs & Corcoran (1952) have estimated that in the waters off the coast of California living cells form only 1·5–4% of the total colloidal or otherwise finely particulate organic matter, called leptopel, and its carotenoid content is almost unknown. Earlier, Fox (1937) filtered off detritus and microplankton from 4000 l. of sea water and extracted from the deposit 0·1 mg of xanthophylls and 0·02 mg of carotenes. Marine muds are also rich in carotenoids (Fox, Updegraff & Novelli, 1944), especially carotenes. This part of their diet could, therefore, provide by conversion at least part of the vitamin A found in euphausiids.

Be it as it may, Euphausiacea seem unique in their ability to accumulate vitamin A in the sea and the interesting possibility remains that they, of all Crustacea, may be able to utilize the ubiquitous crustacean pigment, astaxanthin, as a precursor of vitamin A. The joint presence in the euphausiid eye of large amounts of vitamin A and astaxanthin in similar proportions in different size-groups, as indicated in Figs. 1 and 2, strongly suggests a close interrelationship of these substances in the carotenoid metabolism of euphausiids.

The Development Commission has continued to support our work. We are much indebted to Mr E. Ford and his staff at Millport for making arrangements for, and to Captain R. E. Souter and the crew of M.F.V. *Calanus* for their invaluable assistance in, the collection of specimens. We are grateful to Mr F. S. Russell, F.R.S., at Plymouth and Mr R. E. Savage at Conway and their staffs for the supply of *Crangon vulgaris* and to Mr R. D. Waugh and his staff of the Oyster Research Station, Burnham-on-Crouch, for allowing us facilities for collecting specimens. Our thanks are due to Messrs Lyons Laboratories, Hammersmith, and their chemists, Dr E. B. Hughes and Mr D. H. F. Clayson, for their co-operation in providing us with the lobster eyes. Dr M. Parke of the Plymouth Laboratory kindly supplied the pure cultures of diatoms. We have had much analytical assistance from Mrs W. M. Henschel.

SUMMARY

Results are given for measurements of fat, vitamin A and carotenoids in groups of *Meganyctiphanes norvegica*, *Thysanoessa raschii*, *Crangon allmani*, *C. vulgaris*, *Nephrops norvegicus* and eyes of *Homarus vulgaris*, collected at regular intervals for a year or longer.

In both species of euphausiids vitamin A was consistently present mainly in the ester form and concentrated chiefly in the eyes. More than half the total astaxanthin was in the eyes. No other carotenoids were detected.

Astaxanthin and vitamin A were accumulated by the euphausiids more rapidly during the spring and autumn-feeding periods associated with diatom outbursts than at other seasons. There was a delay in *Thysanoessa raschii*, but not in *Meganyctiphanes norvegica*, between resumption of more rapid growth during spring and autumn and increase in concentration of vitamin A and astaxanthin. The difference was possibly due to differences in diet of the two species in relation to the seasonal biological composition of the plankton.

In the euphausiids, concentrations of vitamin A and astaxanthin were much

higher in larvae and in adults over 30 mm long of *M. norvegica*, and in larvae and adults over 15 mm of *Thysanoessa raschii*, than in mature adults of *Meganyctiphanes norvegica* of 20–30 mm and *Thysanoessa raschii* of 13–15 mm, respectively. An inverse relationship between fat and vitamin A concentration was noted in both species throughout the year.

In *Crangon* spp. and *Nephrops norvegicus* there was no evidence of any seasonal variation of either vitamin A or carotenoids. Differences in the carotenoid metabolism of the two species of *Crangon* are discussed.

In the eyes of *Homarus vulgaris*, vitamin A and carotenoids reached their highest concentrations in the spring, gradually decreasing during the rest of the year.

The metabolism of vitamin A and carotenoids in euphausiids and other Crustacea is considered. The origin of the large reserves of vitamin A in the eyes of euphausiids is discussed, and special attention is given to the importance of astaxanthin as a possible precursor.

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J. Mar. biol. Ass. U.K. (1954) 33, 613–625
Printed in Great Britain

FURTHER NOTES ON A MARINE MEMBER OF THE SAPROLEGNIACEAE, *LEPTOLEGNIA MARINA* N.SP., INFECTING CERTAIN INVERTEBRATES

By D. Atkins, D.Sc.

(Text-figs. 1–5)

Twenty-five years ago I published a note (Atkins, 1929) on a then unnamed member of the Saprolegniaceae, a destructive fungal parasite of the pea-crab, *Pinnotheres*; the name *Leptolegnia marina* is now proposed for this fungus.

SEXUAL REPRODUCTION

In the previous note what appeared to be young oogonia were figured (Atkins, 1929, fig. 5), but sexual organs were not then identified with certainty. In 1943, while preserved material of *Pinnotheres pisum* was being re-examined, the eggs beneath the abdomen of one female were seen to be crowded with round bodies, which proved to be the oogonia and oospores of a fungus (Fig. 1). Identification of these plants with the fungus previously described rests on the presence of that fungus in its asexual reproductive stage in the crab bearing the eggs, and in one of a cluster of sixty-eight eggs otherwise containing plants in the sexual stage. In some eggs containing few oospores the mycelium had in part been emptied, following the formation of zoospores, by short emission tubes resembling those of *Leptolegnia marina*, but definite proof that the oospores are the sexual organs of that species would need evidence from pure cultures. This was the only occurrence of the sexual stage of the fungus in more than 100 infected crabs.

The crab taken on 6 August 1928 from a *Mytilus edulis*, received 6 days earlier from the Camel Estuary, near St Issey Cliff, Padstow, north Cornwall, and which had been kept in a finger-bowl of sea water changed at intervals, was noticed to be moribund on 23 August. The cause of death was infection by *Leptolegnia marina*, the gills being crowded with hyphae. In one gill the mycelium was already empty except for a few cysts, in the others production of zoospores was proceeding; no oogonia were present. The crab was fully berried when taken from the mussel. The eggs, brown in colour, contained advanced embryos, most of which hatched before the death of the crab. The eggs remaining were dirty and degenerate, with a rich growth of bacterial filaments, while many bore clusters of vorticellids. Nearly all of them contained the oogonia and oospores of the infecting fungus, up to 200 in a single egg. Most plants with sexual organs had not previously produced zoospores; this was evident from the absence of projecting tubes on the surface of the *Pinnotheres* egg.

From 6 to 23 August 1928 the temperature of the laboratory sea water was about 16–17° C.

When the ovum of *Pinnotheres pisum*, which is only about 0·3 mm in diameter, is packed with a mass of tangled hyphae and with oogonia, it is exceedingly difficult to tease out the oogonia with mycelial connexions intact, so as to be able to discern the disposition of the sexual organs and especially the origin of the antheridia. That the fungus had attacked eggs containing pre-zoeae added to the difficulty, for the cuticle of these resembled the wall of empty hyphae. The hyphae tended to keep within the cuticle of the zoeae, even of the appendages.

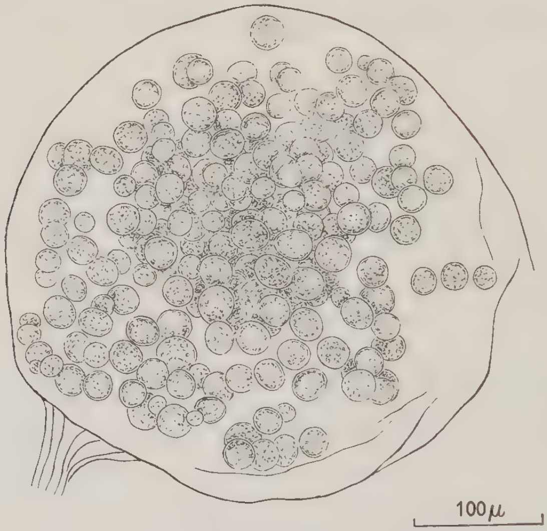

Fig. 1. *Leptolegnia marina*. Immature oospores, thin-walled and with food reserve in scattered drops or granules, within the egg of *Pinnotheres pisum*. Unstained, in 10% formalin.

In some *Pinnotheres* ova the mycelium was young and the oogonia immature (Fig. 2A–F). Other eggs contained plants with numerous oogonia in various stages of maturity. In these the mycelium was mainly empty, but where a few short lengths contained protoplasm these bore young oogonia. In yet others the mycelium was entirely empty and all oospores were ripe (Fig. 3). Septa were frequent.

Oogonia are borne on short side branches (Fig. 2B, E, F), sometimes sessile in diverticula of the hyphae (Figs. 2A; 3D, E), sometimes terminal (Fig. 2C). Fig. 3D is reminiscent of Butler's fig. 19 pl. V (1907) of the sexual apparatus, of *Pythium rostratum*. In *Leptolegnia marina* the frequently sessile position of the oogonium on the hypha may be due to the restricted condition of growth in the *Pinnotheres* ovum. The oogonia are smooth, thin-walled and spherical, although some tend to be pyriform or oval and an occasional one is irregular in shape. Very rarely there may be one or two papillae above the oogonium.

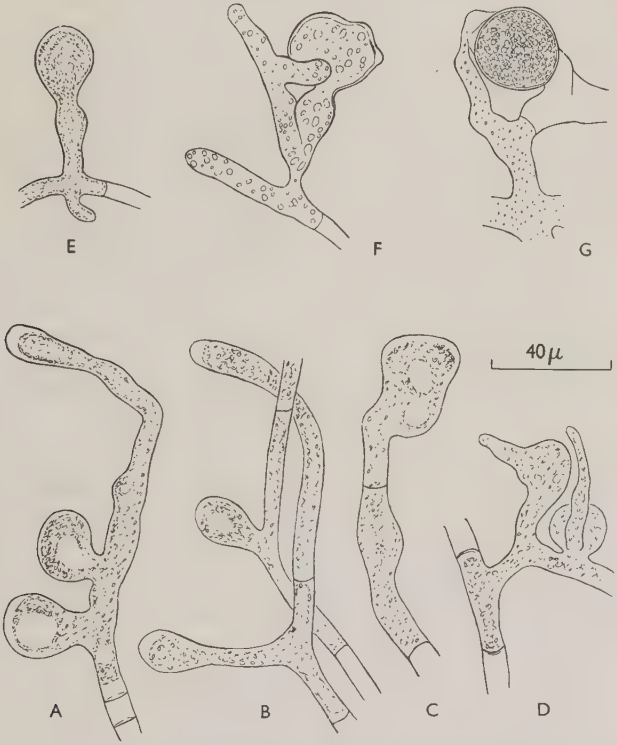

Fig. 2. *Leptolegnia marina*. Young oogonia and immature oospore. In E and F the swelling in the stalk below the oogonium is probably the initial of an hypogynous antheridium. Unstained, in 10% formalin.

Each oogonium contains a single oosphere which fills it completely, and measures 17·5–30μ and occasionally up to 37μ in diameter. The antheridia are hypogynous (Fig. 3A, D, E); those seen were mostly empty. The oogonium is produced into a slight beak where the hypogynous antheridium is applied, and such a structure has not been seen in any other position. It would appear that the antheridia are not always cut off by septa when the oogonium is sessile (see Fig. 3E). Diclinous antheridia have not been observed near young oogonia in the present material and I am not satisfied as to the interpretation of the portions of empty hyphae seen attached to the distal end of a number of oogonia containing ripe oospores, as, for instance, in Fig. 3E. They may be the remains of elongated protrusions similar to those described on the oogonia of *Leptolegnia subterranea* by Harvey (1925), or, more probably, merely a continuation of the growth of the hypha beyond the oogonium, the latter being intercalary; this is suggested by the formation of the oogonia in Fig. 2D. The oogonium depicted in Fig. 2G appears to have an androgynous antheridium,

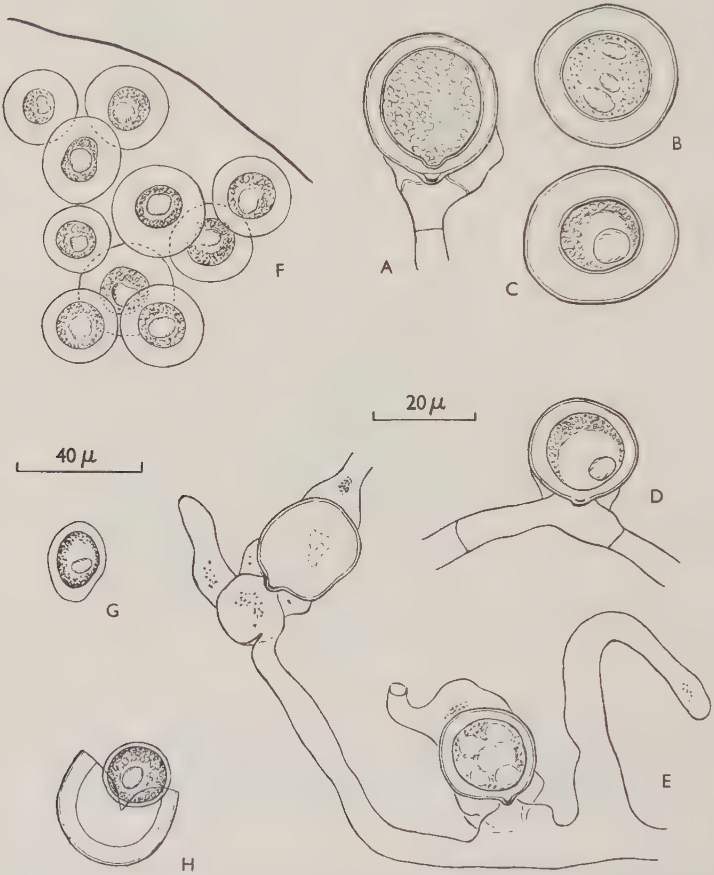

Fig. 3. *Leptolegnia marina.* A: oogonium with oospore and hypogynous antheridium: food reserve scattered. B and C: oospores containing three and one reserve food bodies respectively. D: sessile oogonium with mature oospore and hypogynous antheridium. E: two sessile oogonia on the same branch, both with hypogynous antheridia and both with a portion of hypha of problematical nature attached apically (see pp. 615–6). One oogonium is empty and the oospore in the other is highly vacuolated and perhaps abnormal. Small collections of shining residual granules are present in otherwise empty hyphae. F: fully mature oospores lying beneath membrane of *Pinnotheres* egg. G: pyriform oospore. H: oospore burst by pressure on cover-slip. Unstained, in 10% formalin. A–E to scale on right, F–H to scale on left.

but it may be merely a hyphal branch passing behind the oogonium, and certainly most of the oogonia seen had clearly hypogynous antheridia.

When the oospore is immature the wall is thin (Fig. 2G). As it matures the protoplasmic contents become reduced and the wall thickens greatly (Fig. 3); in fully ripe oospores it is about 7·5μ thick, and is two-layered. The extremely thick outer layer is smooth, colourless, transparent and very faintly stratified, too faintly to be shown in the figures. When oospores are burst by pressure on the cover-slip, sometimes the contents emerge entire, surrounded by a thin wall, the endospore (Fig. 3H). The thin oogonial wall is still distinguishable. In the immature oospore with thin wall, the fat or oily reserve is in scattered droplets (Figs. 2G; 3A); the mature oospore contains one to three reserve bodies, probably when fully mature there is but one, eccentric in position (Fig. 3C, D, F–H). In some oospores this has the appearance of a refringent body, in others of a large oil drop.

The specific characters of *L. marina* are given on p. 622.

ADDITIONAL RECORDS OF OCCURRENCE

In the Body, Eggs and Embryos of Pinnotheres pisum *and in the Body of one Individual of* Pinnotheres pinnotheres (=veterum)

In a previous paper (Atkins, 1929) this fungus was recorded as occurring in crabs taken from *Mytilus edulis* obtained from the Camel Estuary near St Issey Cliff, Padstow, north Cornwall, on 25 November 1927, 24 February, 25 April, 1 August and 14 September 1928. Crabs obtained from *Mytilus* from the same locality on 11 October 1928 were apparently free from infection. The disease was also recorded in crabs taken near the junctions of the Tamar and Tavy (Weir Point, on the Cornwall–Devon border) on 22 March, 10 and 20 April 1928; and from the Yealm Estuary, S. Devon, on 21 July 1928. Subsequently the fungus was again found in female *Pinnotheres pisum* from Padstow mussels received on 27 February, 6 June and 9 August 1929. These mussels were opened over a period of days: the crabs taken from them, and in particular the adult females, were mostly killed and examined the same day, or within a few days, of being found. This rapid examination no doubt explains the small number of crabs in which the fungus was found as compared with previous batches which had been kept together in bowls for several weeks and probably infected each other. In some crabs the fungus was only discovered when they were being searched for an entoniscid.

In June 1952 *Leptolegnia marina* developed in eggs of *Pinnotheres pisum*, ovigerous when obtained from Conway, North Wales; as the eggs were kept in filtered sea water, they probably brought the disease with them.

As stated previously (Atkins, 1929, p. 204), in some few instances it is certain that the crabs were already infected when taken from mussels on arrival from the beds. One instance may be given: among mussels received from Padstow at midday on 1 August 1928, one which was opened the same day contained an adult female carrying a few dead and degenerate eggs. By 2 August the crab was dead, the gills of the left side opaque and invaded by the fungus.

Information on the rate of development of the fungus and the length of time before death of the host ensues is lacking. The time varies no doubt with the disposition of the fungus in the body of the host: once it has invaded the gills then death is rapid. Death is probably more rapid under laboratory than under natural conditions.

A few crabs have shown the infection and have died within 8 days of reaching the laboratory, but more have died of the disease between the 15th and 57th days. In an exceptional instance one died after 78 days in the laboratory. For some time before death occurred this crab had been a milky orange colour, owing to the breaking down of the gonad among which hyphae were found; there were also patches of fungus in the abdomen, and hyphae were in the pleopods and the gills. Whether crabs could harbour the vegetative stage of the fungus for such a length of time before it became fatal seems doubtful, but they may have carried resting bodies caught in hairs, which germinated after the crabs reached the laboratory. On the other hand, it is possible, or probable, that the fungus is widespread and that some crabs became infected in the laboratory tanks. More females than males have been observed to be infected but more females than males have been examined, the proportion of female to male *P. pisum* in a total of 508 taken from mussels received on 27 February, 6 June and 9 August 1929 being approximately four to one.

Leptolegnia marina in vegetative and spore-producing stages has been found in crabs in the laboratory in all months of the year, except February and November, when its absence was probably due to chance. The temperature range of the laboratory sea water was between 8 and 17° C.

The only occurrence of infection of *Pinnotheres pinnotheres* was of a tiny female (2·1 mm carapace width) obtained on 15 March 1928 from Teignmouth and found dead on 4 May with the fungus visible in the walking legs. It most probably became infected in the laboratory.

In the previous paper, although attention was paid mostly to attacks on the crab itself, an infection was recorded in a few embryos which had hyphae radiating from them (Atkins, 1929). The formation of zoospores was not observed and the fungus was assumed to be that already known, as the crab carrying the embryos later developed the disease.

A few further instances of infection of the eggs of *P. pisum* by *Leptolegnia marina* have been observed, namely two egg-masses of 6 June 1929 and one of 1 September 1930. A number have probably been overlooked, for re-examination of preserved material in 1943 revealed the presence of the fungus in the egg-mass of two more crabs; in one the fungus had produced numerous oogonia, as already described.

The following observations were made in September 1930 on the living fungus in *Pinnotheres* eggs. The unbranched hyphae radiating from some of the eggs were up to 600μ long and 7·5–10μ broad, those within the egg were generally broader, 10–40μ. Spore formation was observed in fine external hyphae: the contents divided into a single row of more or less rectangular blocks, which gradually lost their angles and escaped from the small aperture at the tip of the hypha as pear-shaped, biflagellate zoospores. In the intramatrical sporangia the spores were in more than one row. As in the fungus in the crab itself, sporangia were formed from unchanged hyphae.

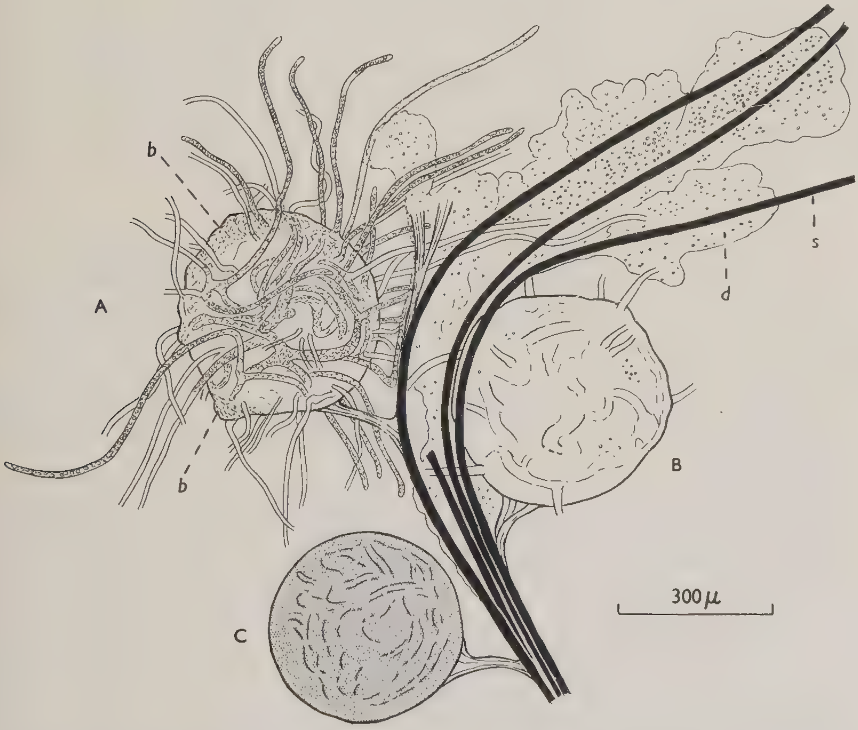

Fig. 4. *Leptolegnia marina.* Three ova of *Pinnotheres pisum* attached to the setae of a pleopod and all infected with the fungus. A: ovum with considerable extra-matrical growth of the fungus. The intra-matrical hyphae are clearly visible as the contents of the ovum have been mostly absorbed. Some hyphae contain zoospores, others are empty after their discharge. B: ovum with mycelium intra-matrical and entirely emptied by the formation of zoospores; the efferent hyphae are all short. C: ovum with mycelium entirely intra-matrical; no efferent hyphae yet formed: ovum opaque as indicated by stippling. *b*, bacteria; *d*, flocculent matter caught on setae (*s*), and in which are embedded numerous encysted spores of the fungus, some of which are indicated. Unstained, in 70% alcohol.

The formation of zoospores was very prolific, and collections of encysted spores on the crab eggs reached a size of 160 by 100μ. Spores also collected in piles on pleopod hairs; these reached a size of 700 by 300μ. It seems improbable that in the event of primary infection taking place soon after the extrusion of the crab eggs any would escape destruction.

There appeared to be a greater tendency to the formation of long discharge tubes when the egg was the substratum than when the crab itself was the host; many infected eggs, however, showed only short discharge tubes. Of plants in two adjacent eggs one may have all efferent hyphae short, while the other may have extra-matrical hyphae of considerable length (Fig. 4, p. 619), so that apparently external conditions are not responsible for the difference. But it should be noted that of the two plants figured, the one with short external hyphae was emptied of contents, while the other was in the midst of zoospore formation. Growth of the fungus is rapid, and it is possible that the external conditions had changed between the emptying of the first plant and external growth of the second. Fouling of the surface results in the formation of long discharge hyphae, as seen on pieces of mantle of *Cardium echinatum* (see p. 621).

In Two Lamellibranchs, Barnea candida *and* Cardium echinatum

On 25 April 1934 one specimen of *Barnea candida* and three of *Cardium echinatum* were examined; they had originally come from Torquay but had been in the Plymouth Laboratory for about 10 weeks; infection may therefore have occurred in the tanks.

In *Barnea candida* the hyphae of a fungus were found in the subfilamentar tissue of the gills; it appeared to be *Leptolegnia marina*, but was not further investigated.

Two of the three *Cardium* were in good condition, the third was gaping, had indeed been gaping for some days and the protruding red foot showed a number of white pustules (Fig. 5A), while similar patches were present on the mantle edge of both valves.

By 30 April this *Cardium* was moribund. The fungal infection had made great progress. The foot was almost covered with a white flocculent layer, which, however, was largely composed of bacteria. By 1 May the *Cardium* was dead. No fungus was found in the gills, although it was present round the point of their attachment to the siphons. In the mantle the tissue surrounding the pustules was invaded by a network of hyphae, but there was little extra-matrical growth. On the foot the extra-matrical hyphae reached a length of about 150μ only.

In the mantle edge broad vegetative hyphae were present, such as previously described and figured from the roof of the gill chamber of *Pinnotheres pisum* (Atkins, 1929, p. 206, fig. 2, p. 205). The hyphae farthest away from those discharging zoospores contained clear, transparent cytoplasm, with shining droplets, probably oil globules.

The fungus lived for about 12 days in mantle fragments placed on 1 May in dishes of sea water; by the end of the period the hyphae had become emptied of their contents by zoospore formation. By 5 May bacteria coated the fragments to some depth, and, apparently because of this fouling, the external hyphae were unusually long, up to about 400μ in length, allowing the zoospores to be liberated beyond the bacterial layer (see Fig. 5B). From 7 May onward there was a great tendency for rounded masses and short lengths of protoplasm to remain when the rest of the hypha had emptied its contents in zoospore formation. Each of these bodies then sent out a hypha which eventually penetrated the wall of the old hypha; they were not thick-

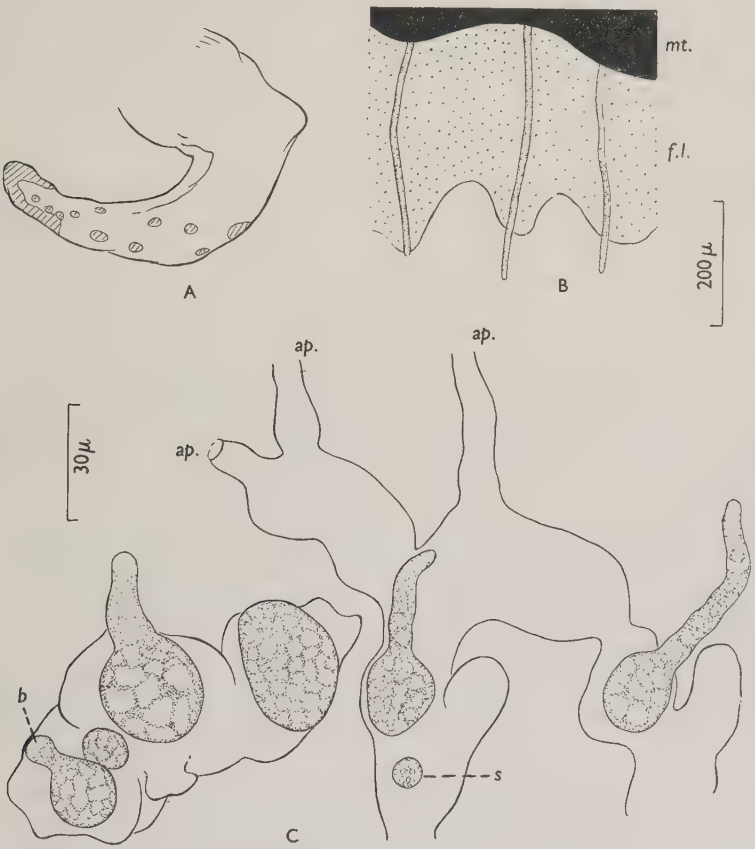

Fig. 5. *Leptolegnia marina* infecting *Cardium echinatum*. A: sketch of areas of infection on foot of the *Cardium*. B: efferent hyphae extending from the mantle (*mt.*) and passing through a flocculent layer (*f.l.*) composed of mucus, sloughed off cells, protozoa, bacteria, etc. C: unusually coarse hyphae (in mantle) most of the contents of which have been used up in zoospore formation, but with a number of rounded bodies of vacuolated protoplasm remaining. Several of the bodies have sent out hyphae; note bulge of tip (*b*) where pressed against wall. *ap.* aperture through which zoospores from old sporangium have been released; *s*, encysted spore. From living material.

walled, and possibly were vegetative portions reconstituting themselves for renewed growth (Fig. 5C).

The liberation of biflagellated pear-shaped zoospores was seen a number of times during the 12 days the fungus lived in the mantle fragments. The sporangia were formed from unchanged hyphae; the number of spores in a row varied with the width of the hyphae, from one to several. The zoospores were about 13μ long; they were observed to remain active for up to 30 min before encysting: encysted spores were 6–10μ in diameter. These measurements agree well with those of *Leptolegnia marina* of *Pinnotheres*, except that no small zoospores were seen. No sexual reproductive organs were identified with certainty.

It is considered that the fungus in the two lamellibranchs was *Leptolegnia marina*.

SPECIFIC CHARACTERS

Leptolegnia marina n.sp.

Mycelio ex norma intramatricali, profuse ramoso, formato de hyphis 7·5–20 μ [40 μ] latis; sporangiis filamentosis, intramatricalibus, de hyphis immutatis formatis, subinde proliferosis; sporis in una vel pleribusque lineis (de latitudine sporangii); zoosporis in primo statu motili, pyriformibus, tum 6–11 μ latitudine cum in capsulas inclusa sunt.

Generatione sexuali rara, per abundantia oogonia, intramatricalia, sphaeralia vel ovalia, cum parietibus teneribus, paulim rostrata ad antheridium adiunctum; antheridiis plerumque hypogynosis; oosporo unico, lato 17·5–30 μ [37 μ], complenti totum oogonium, cum pariete glabro, incolorato, diaphano, crasso 7·5 μ, de duobus stratis composito; oosporo maturo continenti 1–3 (ferme 1) magna corpora reservata; germinatione non observata.

Mari incolens; parasitica in quibusdam animalibus invertebratis marinis; in corpore, in ovis et in embryonibus *Pinnotheres pisum*, in corpore *P. pinnotheres* (=*veterum*), et in duobus molluscis lamellibranchiatis, *Barnea candida* et *Cardium echinatum*.

The characters of *Leptolegnia marina* (described in Atkins, 1929, and in the present paper) may now be summarized as follows.

Mycelium almost entirely intra-matrical and then freely branched. Hyphae 7·5–20 μ in diameter, sometimes up to 40 μ in diameter. Sporangia filamentous, formed from unchanged hyphae within the tissue of the host; occasionally proliferous: short branches for the discharge of zoospores lead to the exterior of the host. Number of rows of zoospores within the sporangium vary with its width, from one to several. When active within the sporangium the zoospores may be oval, pyriform or rod-shaped. First zoospores 8–14 μ in length, pyriform—when free swimming—biflagellate, swimming away upon emergence and encysting after an interval varying from a few to 50 min. Encysted spores 6–11 μ in diameter. Is probably diplanetic (Atkins, 1929, pp. 213–14). Sexual reproduction rare, then oogonia abundant. Oogonia formed within tissue of host: borne on short side branches, frequently sessile in diverticula of the hyphae, perhaps intercalary, generally spherical, sometimes pyriform or oval, rarely irregular in shape; thin-walled, smooth, very occasionally with one or two small papillae. A slight beak present where hypogynous antheridium attached. Antheridia generally hypogynous. Oospores single, completely filling oogonium, 17·5–30 μ in diameter, occasionally up to 37 μ in diameter. Wall smooth, colourless, transparent, exceedingly thick (up to 7·5 μ), two-layered, very faintly stratified and all surrounded by oogonial wall. Mature egg with 1 to 3, generally one, large eccentric oil or fat body. Germination not observed.

Habitat: marine, parasitic in certain marine invertebrates, being so far known from the body, eggs and embryos of *Pinnotheres pisum*, from the body of *P. pinnotheres* (=*veterum*), and from two lamellibranchs, *Barnea candida* and *Cardium echinatum*.

AFFINITIES

The saprolegniacean fungus which attacks certain marine invertebrates, is considered to belong to the genus *Leptolegnia* with which it has the following characters in common: (i) sporangia filamentous, formed from unchanged hyphae; (ii) zoospores on emerging swim away; (iii) single oospore completely filling the oogonium, eccentric; (iv) ripe oospore wall exceedingly thick. (It differs from the genus *Saprolegnia* in characters i, iii and iv.)

Leptolegnia marina is distinguished from the three known species of the genus, *L. caudata* de Bary, *L. subterranea* Coker and Harvey and *L. eccentrica* Coker in its marine habitat, in which indeed it differs from all known members of the Saprolegniaceae,[1] although one member *Synchaetophagus balticus*, parasitic in the rotifer, *Synchaeta monopus*, has been recorded from brackish water, salinity 5·3–11‰, in the Baltic (Apstein, 1911). *Leptolegnia marina* is also peculiar in that the mycelium is almost entirely intra-matrical, although when *Pinnotheres* eggs are the substrata there is some tendency to extra-matrical growth, not exceeding 1·0 mm in length, the external hyphae being slender, 7·5–10μ thick, and rarely branched, with zoospores in a single row.

The mycelium of *Leptolegnia marina* is not only more freely branched than in the other three species, but is of somewhat coarser growth (7·5–20μ in diameter, sometimes up to 40μ), although Couch (1932, p. 584) records that strains of *L. caudata* vary considerably in their robustness, the hyphae from some often approximating a well-developed *Saprolegnia* in thickness and length. In *Leptolegnia caudata* the hyphae are about 10–18μ thick (Coker & Matthews, 1937, p. 29), in *L. subterranea* 9·4–11·8μ thick (Harvey, 1925, p. 158) and in *L. eccentrica* 4·8–7·2μ thick (Coker, 1927, p. 215).

L. marina differs from the other species in that the zoospores are formed typically in more than one row in the sporangium, the number varying with its width from one to several. In *L. caudata* and *L. subterranea* the zoospores are said to be typically in a single row (Coker & Matthews, 1937, pp. 29, 30), from which it would appear that they are not invariably so. The peculiar bending back or reshaping of the spores before swimming away, which is characteristic of the other species (Petersen, 1910; Coker, 1923, 1927; Harvey, 1925; Mathews, 1932) was not observed in *L. marina*.

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[1] Since this was written a second marine member of the Saprolegniaceae, *Plectospira dubia* n.sp., has been found (Atkins, 1954).

In *L. caudata* the antheridia are diclinous (Coker, 1923, p. 158; Couch, 1932, p. 596; Coker & Matthews, 1937, p. 30), in *L. eccentrica* closely androgynous (Coker, 1927) and in *L. subterranea* lacking (Harvey, 1925), while in *L. marina* they are generally hypogynous.

L. marina agrees with *L. caudata* in being a destructive parasite of an animal, but the latter species is also found saprophytic on animal and vegetable substrata (Couch, 1932, p. 584). It moreover agrees with *L. caudata* in the occasional renewal of the sporangium within an empty one (for *L. caudata* see Coker, 1923; Coker & Matthews, 1937; and for *L. marina* see Atkins, 1929); in the rarity of sexual reproduction (for *L. caudata* see Petersen, 1910; Coker 1923; Couch, 1932) and the formation of oogonia within the tissue of the animal host (for *L. caudata* see Petersen, 1910, p. 513).

In *L. marina* the greater part of the protoplasm of the mycelium may be absorbed in zoospore formation (Atkins, 1929, pp. 209–210), agreeing in this with *L. caudata* (Petersen, 1910) and *L. eccentrica* (Coker, 1927). As in *L. eccentrica* and *L. subterranea* so in *L. marina* spore discharge occurs commonly at night.

L. marina in the great thickness of the mature oospore wall (up to $7{\cdot}5\mu$ thick) resembles most nearly *L. eccentrica* (5μ or more, Coker, 1927) and *L. subterranea* ($3{\cdot}7$–$5{\cdot}5\mu$, rarely $6{\cdot}5\,\mu$, Harvey, 1925) but differs from them in that the wall is smooth. The oospore wall is stated by Couch (1932) to be very thick in *L. caudata*, but he gives no measurements: according to Coker (1927) it is only about one-third as thick as in the other two species.

In *L. marina* the food reserve in the fully mature oospore is in the form of a single large eccentric refringent body or oil drop, thus agreeing with *L. eccentrica* (Coker, 1927) and differing from *L. caudata* (Coker, 1923; Couch, 1932) and *L. subterranea* (Harvey, 1925) in both of which it takes the form of a lunate cap of small oil drops.

I gratefully acknowledge the kindness of the late Prof. J. H. Orton, F.R.S., Dr W. B. Turrill, Miss E. M. Wakefield and the late Miss M. A. Sexton in procuring for me excerpts from the *Journal of the Elisha Mitchell Scientific Society* which I was unable to obtain on loan. Mrs A. H. Beers of the Botany Department, University of North Carolina, kindly supplied me with references to descriptions of two species of *Leptolegnia*. I wish to record my appreciation of the great help the Surrey County Library—with its associated network of libraries—has been to me in supplying on loan many necessary publications.

I am indebted to Dr W. R. G. Atkins, F.R.S., for the interest he has taken in this marine fungus, and to Dr B. Barnes for reading the manuscript. Dr J. Morton has most kindly turned the diagnosis into Latin.

Type material has been deposited in the British Museum.

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J. Mar. biol. Ass. U.K. (1954) 33, 627–636
Printed in Great Britain

LEG DISPOSITION IN THE BRACHYURAN MEGALOPA WHEN SWIMMING

By D. Atkins, D.Sc.

(Text-figs. 1 and 2)

As long ago as 1852 Dana carefully described and figured the position of the legs of certain megalopae whilst swimming. Later writers having apparently overlooked his observations, his remarks are worth quoting in full, remembering that he is writing of five pairs of legs: 'In the genera *Monolepis*, *Marestia* and *Cyllene*, and probably also in *Tribola*,* the posterior legs are capable of overlying the posterior angle of the carapax, and there is a corresponding depression in this surface, which depression is sometimes abrupt and channel-like. The penult pair of legs also admits of being thrown forward over the border of the carapax, and extends above the base of the eyes, so that the tarsus hangs down in front. The second and third pairs fold up and partially overlap the sides of the carapax, beneath the fourth or penult pair, or, as is sometimes seen, the third pair is thrown forward like the fourth. A species very near *Megalopa mutica*, abundant near the Cape of Good Hope is figured on plate 31, with the legs in the position they had *while the animal was swimming*. This position is not often observed, since the animal when disturbed, is almost sure to swim with the legs extended; and the hinder legs are mostly like the others in form and habit. On the same plate, this position is shown for another species from the Sooloo Sea; in the former, the surface of the carapax is simply a little depressed or concave for the folded posterior legs (genus *Marestia*) while in the latter (genus *Monolepis*), as shown in figure 5*b*, there is an abrupt channel' (Dana, 1852*b*, pp. 485–6, pl. 31, figs. 2*a*, 5*a*).

Unaware of Dana's work I have recently made observations on the swimming of the megalopae of a number of species of crabs at Plymouth.

The brachyuran megalopa when walking (e.g. *Carcinides*), or when clinging to an object (e.g. *Macropodia*), uses its legs as in the crab stage. When swimming, by means of the pleopods, with abdomen outstretched, two different methods of disposing of the legs have been observed. In the more spectacular method, found in all but three of the species examined at Plymouth, the legs are packed away in a curious manner so that the body is compact and the outline approximately rectangular in dorsal and ventral view, but wider in front than behind because of the eyestalks. This arrangement of the legs when swimming is common to the following species: *Portunus depurator*, *P. puber*, *P. arcuatus*, *P. pusillus*, *P. marmoreus*, *P. holsatus*, *Carcinides maenas*, *Portumnus latipes*, *Cancer pagurus*, *Atelecyclus septemdentatus*, *Pilumnus hirtellus*, *Pinnotheres pisum* (but not *P. pinnotheres*) (Brachyrhyncha); *Ebalia tuberosa*, *E. cranchii* (Oxystomata); *Maia squinado*, *Eurynome aspera*, *Hyas* sp., and several species of *Inachus* and *Macropodia* (Oxyrhyncha). It is briefly that, while the chelipeds and first two pairs of walking legs are folded beneath the body, the third and fourth pairs are wrapped over the dorsum, the third being stretched out anteriorly (Figs. 1 and 2).

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* Megalopae respectively of *Ocypode*, *Grapsids?*, *Lupa* (*Portunus*) and *Plagusia?*, see Gurney, 1939, pp. 118, 119.

The chelipeds are carried on the ventral surface, bent upon themselves at the carpus, the chelae pointing posteriorly and touching in the mid-line of the thorax (Fig. 2B). The exact disposal of the chelipeds varies somewhat according to their length and size. In the megalopa of *Carcinides* there is a curiously large hook on the ischium of the cheliped, and when the chelipeds are folded for swimming the chela lies to the inner side of this (Fig. 2A). It appears to be present in most of the British Brachyrhyncha, although not in the Oxyrhyncha and Oxystomata megalopae (Lebour, 1928), and Lebour (1944, p. 13) noted in *Portumnus latipes* that these large hooks help in holding fast the food while the megalopa swims.

The proximal segments of the first and second walking legs are directed anteriorly and dorsally close to the side of the body, even slightly overlapping the dorsum in long-legged megalopae (Fig. 1C), bent sharply at the carpus, the propodus and dactylus being on the ventral surface, lying close together and to the chelipeds (Fig. 2B).

The proximal segments of the third walking legs are curved round the side of the body on to the dorsum and the leg is stretched out anteriorly, fitting in a groove by the side of the gastric hump. The dactylus curves over the eye stalk and, according to the length of the leg, projects slightly beyond it, as in *Eurynome aspera*, *Ebalia* (Fig. 1B); or is looped round it on to the ventral surface, the tip directed posteriorly, as in *Hyas*, *Inachus*, *Macropodia* (Fig. 1C), with long slender legs. In *Carcinides maenas* the dactylus is sometimes stretched out straight and sometimes curved round the eye (Figs. 1A, 2A) as it is also in *Portunus* spp.

The fourth walking legs, which are curved over the dorsum, are bent back upon themselves at the carpus, the ischium and merus being directed anteriorly and medially, the carpus and propodus posteriorly and outward and the dactylus posteriorly and inward, its tip reaching the base of the ischium. The tip projects slightly beyond the thorax, fitting into the angle between it and the abdomen. In *Macropodia* and *Inachus* the proximal ends of the carpi of the two sides touch or almost touch in the mid-dorsal line (Fig. 1C), while there is a small space between them in *Eurynome* and a wider space in

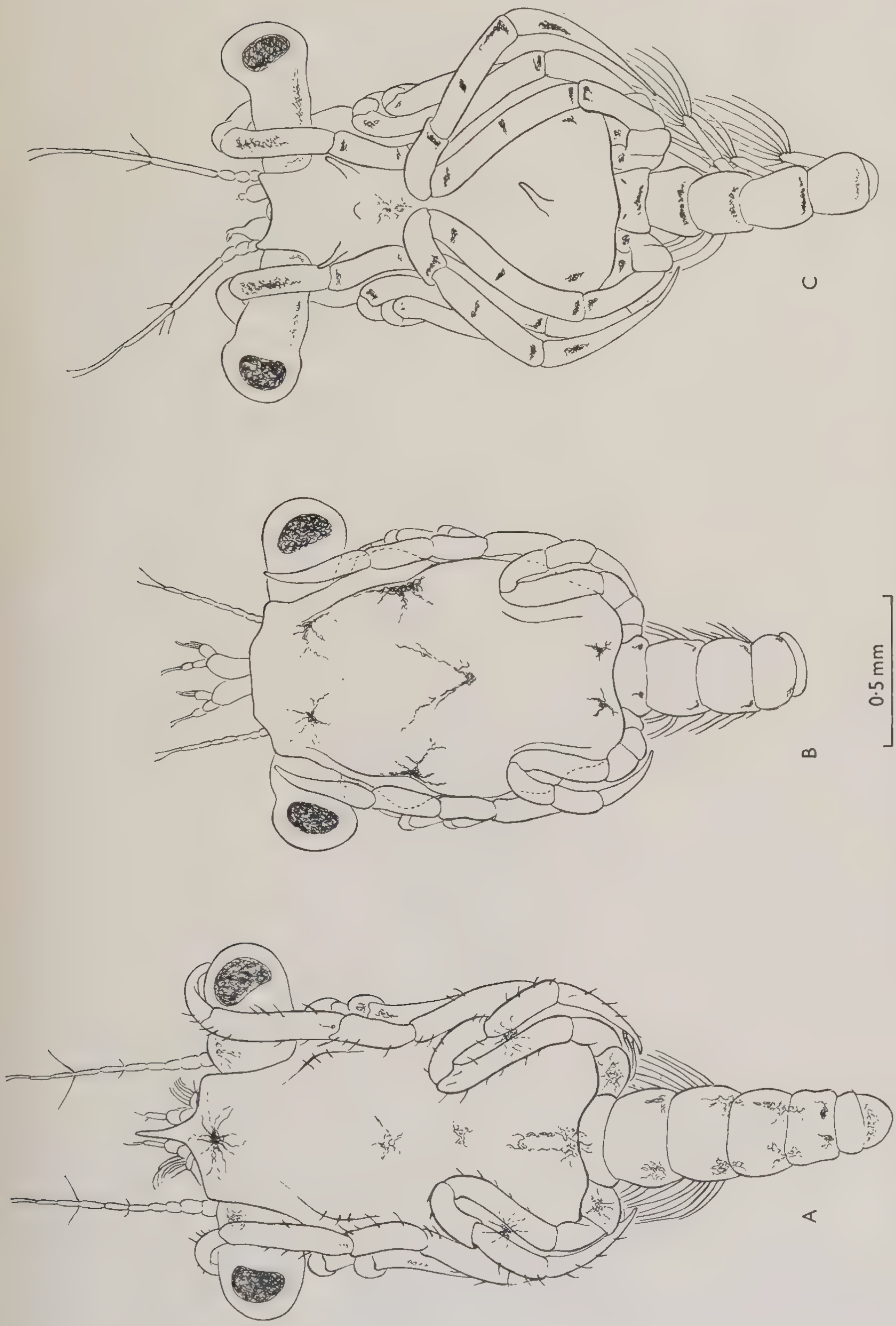

Fig. 1. Megalopae with legs in the swimming position, dorsal view: drawn alive. A, *Carcinides maenas*; B, *Ebalia tuberosa*; C, *Macropodia* sp.

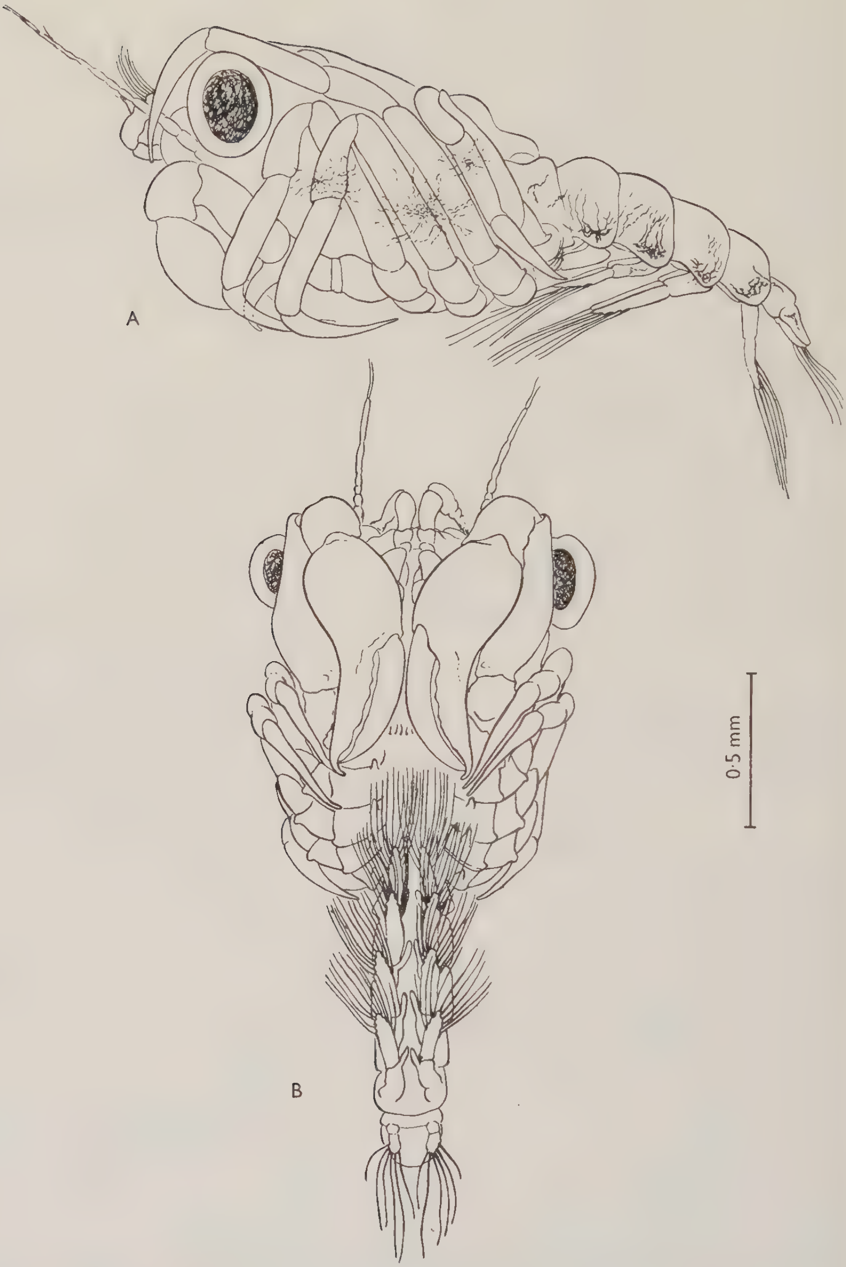

Fig. 2. Megalopae with legs in the swimming position, but being under the influence of ethyl acetate the posterior pair or pairs of pleopods are directed posteriorly. A, *Carcinides maenas*, side view; B, *Ebalia tuberosa*, ventral view.

Portunus, *Carcinides* and *Ebalia*. In *Ebalia* the body is thick and the fourth walking legs fit into depressions, while the third are more to the side than in most megalopae and curve round the dorsal hump (Fig. 1B).

The arrangement of the legs is most easily seen in *Macropodia* and *Inachus* in which the body is nearly transparent and little pigmented, and the legs banded with dark pigment, so that the third and fourth walking legs are conspicuous on the dorsum. Then, perhaps owing to the length of the legs or conditions of captivity, these megalopae and those of *Eurynome*, arrange their legs slowly enough for the eyes to follow the movement. Also some of them were observed frequently to rest on the bottom after a swim with their legs in the swimming position.

It was at first difficult to see the manner in which the *Carcinides* megalopa carried its legs, for the legs are transparent and almost colourless. When the third and fourth walking legs are folded over the back they are practically invisible. Moreover, these megalopae are particularly rapid in their movements, folding the legs and jumping into their swim in an instant. The forward and upward leap is coincident with the sudden straightening of the abdomen from the bent position beneath the thorax.

Unlike Williamson's (1903, p. 158) observation that in the megalopa of *Carcinides* the abdomen remains extended and is never tucked in under the thorax, the few specimens seen walked with it curved beneath the thorax; when molested the animal curled up with the legs in the swimming position and the abdomen tucked under the thorax, taking up the position shown in Crane's figure 2 (1940) of *Ocypode albicans*. Several other species behaved in the same manner (see also Kemp, 1915, p. 219): it was recorded of *Calappa marmorata* (Oxystomata) by Smith (1880, p. 266), but with the difference that chelipeds and all legs were folded very compactly beneath the carapace. This behaviour possibly varies with age, the abdomen being more apt to be tucked under the body as the moult to the crab stage approaches. The megalopae of *Macropodia* and *Inachus*, on the other hand, were rarely seen with the abdomen bent under, even during the day or two preceding the moult, although they momentarily flexed it. This habitual extension of the abdomen may be because these megalopae rarely seem to walk, but will cling to objects with all legs.

That the described arrangement of the legs when swimming is probably common to many other megalopae than those observed at Plymouth would seem to be indicated by many published figures of this post-larval stage, the proximal segments of the third and fourth walking legs projecting well beyond the carapace, either posteriorly or laterally (Connolly, 1923, fig. 11; 1925, fig. 6; Hart, 1935, figs. 2A, 3K, 6A, 7H; Lebour, 1928, many figures) and some even showing the fourth pair of legs folded over the dorsum, for instance Connolly's figure 11 of the megalopa of *Cancer amoenus* and Lebour's figure 8, pl. xi, of *Xantho incisus*. In some instances, no doubt, the protrusion of the proximal segments of the last two legs, as shown in the figures mentioned above, is partly due to the preservative, especially if formalin had been used, but Dr Lebour informs me that her figures were of living or moribund specimens so that the proximal segments of the last two pairs of legs would be in the normal position.

This comparative freedom of the last two pairs of legs from the carapace as compared with the other legs, which allows of their being wrapped over the dorsum in the swimming megalopa, is more pronounced in some megalopae and crabs than in others. In certain species the last two pairs of legs, which are said to arise dorsally, have a specialized function if not specialized formation, and their freedom of movement is utilized to hold objects over the carapace. For instance in the Dorippidae (Oxystomata), *Ethusa investigatoris* (Menon, 1937), *Dorippe dorsipes* and *D. facchino* (Hornell, 1922, p. 932) have the claws of the last two pairs of legs modified so that they can hold shells, etc., on their backs, and this habit may even be exhibited by the megalopa (Menon, 1937, p. 42). The last two pairs of legs of the first and second young crab stages of *Pilumnus hirtellus* have great freedom of movement and can be stretched over the dorsum when clasping objects. The last pair, moreover, can be stretched directly backward and the young crabs frequently stand on their heads, ventral surface uppermost, supported on the front and the tips of the posteriorly and dorsally stretched last pair of legs. Dressing of the dorsum by the last pair of legs was not observed; it may be that debris collects on it because young crabs so frequently lie on their backs.

Among the megalopae observed at Plymouth three exceptions occurred to the usual method of disposing of the legs. In these, all the legs including the chelipeds were bent up under the body while swimming, as described by Gurney (1903, p. 464) for *Corystes cassivelaunus*. In addition to *Corystes*, *Thia polita* and *Pinnotheres pinnotheres* (=*veterum*) swam in this manner. These megalopae appeared to swim less rapidly than those which were more finely stream-lined. They were obtained by moulting from zoeae in captivity, but seemed healthy enough; they were watched swimming a number of times, and it would appear to be their normal method, although it would have been more satisfactory if the megalopae had been taken direct from the plankton. As Dana (1852*b*, p. 486) has cautioned, megalopae may need to be watched for some time before they show their characteristic method of swimming. Those which normally swim with the third and fourth legs over the dorsum may on occasion swim without packing the legs tightly away, but holding them loosely bent about the middle, with the chelipeds in a loose bow, tips close together in mid-line. They then swim slowly by comparison with their rapid movement when the legs are compactly stowed away.

A reason for the legs being held close to the body, the third and fourth folded over the dorsum, is no doubt that the stream-line effect offers less resistance to movement in water; moreover, the arrangement may have something to do with improved balance whilst swimming. If it allowed of increased speed in swimming it would be an advantage in escaping from danger. Although megalopae are known to eat fast-moving larvae, including zoeae and other megalopae (Lebour, 1927, p. 807; 1928, p. 500), it is not known if they actively hunt them. Incidentally the removal of the last two pairs of legs on to the dorsum prevents any possibility of their interfering with the action of the anterior pleopods, the swimming setae of which extend well forward on to the thorax at the beginning of the effective stroke, which is backward with the plumose setae spread out fanwise. Less effective is the arrangement in which all legs are bent beneath the body, as can be assessed by watching the swimming of megalopae with the two different arrangements of the legs.

The three species of megalopae which swim with all legs folded beneath the body possibly have a pelagic existence of short duration. The megalopa of *Corystes cassivelaunus* is very crab-like and, as noted by Gurney (1903, pp. 463–4), has most of the characteristics and habits of the adult, burrowing into fine sand. According to him it appears not to be a pelagic form properly speaking, and was only once obtained in the tow-net, and then within a fathom or so of the bottom, in deep water. Very occasionally the megalopae have been taken in swarms at the surface (Russell, 1927, p. 602): as the zoeae apparently have a preference for the surface layers (Russell, 1927, p. 602) it is possible that the swarms of megalopae had not long moulted into that stage. The megalopa of *Thia polita* (which incidentally has rather short legs) if provided with muddy gravel, on which the adult is taken, soon backs beneath it: this megalopa also may possibly have a short swimming stage. *Pinnotheres pinnotheres* possibly enters its host (*Ascidia mentula* at Plymouth) in the megalopa stage, for it is of a good size with a carapace length of 0·96 mm. and width of 0·8 mm.; the megalopae of *Pinnixa faba* (Pinnotheridae) are known to do so (Wells, 1940). These megalopae once in the host would feed after the manner of the adult and would be in a protected position. The smaller megalopa of *P. pisum*, with carapace length of 0·69 mm. and width of 0·56 mm. swims with the third and fourth walking legs over the dorsum and because of its small size it is doubtful whether this species enters the mollusc host in the megalopa stage; the risk of its being smothered in mucus and expelled may be too great.

PREVIOUS REFERENCES TO THE DISPOSITION OF THE LEGS IN MEGALOPAE

The megalopae of crabs occurring at Plymouth are of no great size. With the outstanding exception of *Corystes cassivelaunus*, with carapace length 3·36–3·6 mm and width 3·1–3·24 mm, the largest Plymouth megalopa, that of *Atelecyclus septemdentatus*, has a carapace length of 2·7 mm. Although a few references to the position of the legs whilst swimming of megalopae of this size occur (Hyman, 1920, p. 497; 1922, p. 455; Lebour, 1927, p. 807), they are not detailed, merely mentioning that the legs are folded against the body. They do not describe the peculiar posture noted in this paper. Hyman (1920, p. 497), however, mentioned that the last pair of legs in *Uca* (*Gelasimus*) are carried folded over the back and kept in that position even when clinging or walking.

The only account of the peculiar posture as occurring during swimming is that of Dana (1852*a*, *b*) already quoted (see p. 627). He observed it of megalopae of three genera, and of carapace length 3·3–11·0 mm. In addition several detailed descriptions occur of the complicated folding of the legs and of the grooving of the sides of the body and of the carapace posterio-laterally for the reception of the legs, and of the thorax for the repose of the abdomen, in a group of relatively gigantic megalopae, with carapace length 4·0–9·4 mm, with thick hard cuticle, some of which belong to the Ocypodidae (Say, 1817; Smith, 1873; Kemp, 1915; Miyake, 1939, pp. 193–5, as a crab; Crane, 1940; Edmonson, 1949). Most of these descriptions are of museum material, for curiously enough these large megalopae appear frequently to retain their legs folded against the body after death. Except for Dana, who actually observed them swimming, their peculiar characteristics have been considered as adapting them for life on sandy shores where certain of them have been taken, and some are known to moult into the first crab stage (Say, 1817, p. 158; Smith, 1873; 1880, p. 255; Kemp, 1915, p. 219; Edmonson, 1949, p. 235), and where the adults live (Rathbun, 1918, pp. 369, 372, 374). Such are rotund, have thick hard cuticle and the body so grooved for the reception of the legs and abdomen that they may be rolled about like tiny balls without appreciable injury (Crane, 1940, pp. 66, 81; Edmonson, 1949, p. 235). It may be recalled here that the megalopa of *Ebalia*, although tiny with a carapace length of about 1·2 mm, is also rotund and has marked depressions for the reception of the last pair of legs (p. 631): the adult crabs occur in sand. It appears to me that in all probability the stream-lining of the body was originally an adaptation for swimming, for these large megalopae have the pleopods fully developed and a number have been taken in the open sea (Dana, 1852*a*; 1852*b*; Smith, 1880, p. 255; Crane, 1940, p. 81; Edmonson, 1949, p. 243). Moreover, the posture is common to many megalopae the adults of which do not inhabit sandy beaches, and grooving of the body for the reception of the legs has been described in a megalopa of *Plagusia* of carapace length 4·6 mm (Rathbun, 1924; Boone, 1927, p. 271; Crane, 1940, p. 70) the adults of which live among rocks awash at high tide and on drift timber in the open sea (Rathbun, 1918, p. 332). No doubt the grooving of the body for the reception of the legs when swimming is more apparent in large megalopae with thick cuticle than in those with thin cuticle or of small size.

This work was made possible by the kindness of the Board of Studies in Zoology of London University in granting me the use of the London

University Table at the Laboratory of the Marine Biological Association at Plymouth.

I was unable to obtain on loan the volume of plates of Dana's Crustacea U.S. Navy (1852*b*) and am indebted to Dr J. Morton for kindly making sketches from pl. 31 of two megalopae in the swimming attitude.

SUMMARY

Many brachyuran megalopae swim with the legs disposed in a curious manner. The chelipeds and first two pairs of walking legs are folded tightly beneath the body, while the two posterior pairs are wrapped over the dorsum, the third pair being stretched out anteriorly, the dactylus, according to its length, touching or being looped round the eye-stalk. It is suggested that this posture gives stream-lining to the megalopa and improves its swimming. The described arrangement has been observed in all but three of some twenty-five species occurring at Plymouth.

In the three exceptions, *Corystes cassivelaunus*, *Thia polita* and *Pinnotheres pinnotheres* (=*veterum*), all legs are carried folded beneath the body.

Reference is made to previous descriptions of the folding of the legs in the brachyuran megalopa.

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J. Mar. biol. Ass. U.K. (1954) 33, 637–643
Printed in Great Britain

A PENETROMETER FOR USE ON WATER-COVERED BEACHES

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(Text-figs. 1 and 2)

The primary use of penetrometers has been in the road-building industry for testing materials such as tar and asphalt which are virtually homogenous in texture. They have also been used extensively and in wide variety in the food industry to determine optimum textures of products being canned, and an important application has been the determination of the toughness of cheese.

The idea underlying the use of devices to measure resistance to penetration has, of course, been to get away from dependence upon mere qualitative descriptions of firmness, and where interest attaches to investigating that of estuary bottoms and of other grounds covered by modest depths of water, quantitative description has become essential. Only so can conditions at places far apart be acceptably compared.

The importance attached in marine-biological literature to the nature of beaches in connexion with the burrowing powers of animals which inhabit them, has prompted the thought that it might be useful to make known the existence of an instrument which has been successfully used in shallow water to ascertain the firmness of bottoms *in situ*.

It will be convenient in what follows to speak of 'dry beaches' simply in contradistinction to water-covered beaches, by using the expression in the usual hydrographical sense to convey that the falling tide has uncovered them, and not with any connotation of aridity at all. As regards those striking sand properties dilatancy and thixotropy upon consideration of which Kendall thirty years and more ago based his stimulating theory of fossil earthquakes which alone could account for the large and economically important 'wash outs' which occur as amorphous sand infillings in certain coal seams of the West Riding, it does seem that students of sea and sand interrelationships might sometimes make useful small-scale application of Kendall's views on the events due to earthquake shock which can attend the quasi-liquefaction of vast stretches of sand traversed by a watercourse. There surely must be an important gradation of magnitudes between the mammoth physiographical events pictured by Kendall, and those on the micro scale seen by a man who contemplates the aureole of whitening and drying around his feet as he walks on a beach not far from the water's edge, and who finds that if he pats the ground repeatedly he produces a small quicksand.

In this connexion it is suggestive to think of the quicksands of the Solway shore which are in dangerous evidence at times when a rising tide effects a rapid extension of water cover landwards, and of such events as the extensive migration of buoys near the Goodwins which, after remaining firmly fixed for many years, suddenly lose their hold at a time of strong spring tides as very shallow water cover is quickly succeeded by deep. There is abundant reason why ever-increasing attention ought to be given to the study of the firmness and constitution of beach soils both when water-covered and dry.

The interest of the marine biologist in the dilatancy and thixotropy of the sand on beaches wherein animal communities live, has been discussed by Yonge (1950), by Chapman (1949) and by Chapman & Newell (1947).

In the two earliest of these papers there is an account of a device used for measuring the resistance to penetration of marine soils *in situ* on dry beaches.

Use of the small penetrometer in question produced results which were always expressed as the pressures in g/cm^2 necessary to thrust a disc 0·125 cm^2 in area 3 cm deep into a soil. Presumably where attention is limited to recording the resistance to a penetration of such modest amount as 3 cm the data collected can be accepted as measures of the resistance to burrowing which small animals would experience at the same place. It is to be assumed that the users of the small penetrometer just referred to (and apparently little longer than 40 cm overall), found no need to suppose that their results might have been somewhat falsified in point of intended applicability, by sand compaction changes occasioned by their own weight.

The concern of marine scientists with the texture, make-up, and morphology of beaches goes far beyond that of marine biologists of course, and very many factors have to be considered by those who study the potency of wave-sculpting, and the nature and magnitude of other causes of sea erosion, to say no more. Quite often it is necessary to be very chary of applying what is learnt regarding erosion trends, the effects of waves, the magnitudes of sand transports, etc., at one place, to the making of inferences in respect of other beaches even though exposures and wave conditions may be very similar. That very much indeed depends upon the actual make-up and natural compaction of the sands on a beach, is most impressively attested by the remarkable detail with which the Dutch have found it necessary to investigate the grain composition and the mineral constitution of their beach materials.

Where, as with them, the issues are so vitally important, it is risky indeed to assume that information gleaned at one place will necessarily apply adequately elsewhere.

There is in Britain nothing like the school of sedimentology of Wageningen led by Doeglas, nor has our country yet seen beach studies made in anything like the detail of those featuring so much in French marine-geological literature, but there are solid signs of an awakening interest.

It would be of interest (were this the place) to consider the findings of

Emery (1945) on the effects of the entrapment of air in different grades of beach sand, and those of Trask & Rolston (1950) regarding the relation of sediment strength to water content and grain size. Most of the considerations which were in mind when the large penetrometer to be described was designed, are set out in an excellent article by Kindle (1936). He describes beaches which exhibit all degrees of firmness and lack of it within the distance of a fraction of a mile, and dwells upon the abruptness of the transition from firm to soft sand which often exists. He cites such a contrast in the case of an English beach at Skegness, and gives a discussion of the effects upon beach sand firmness as the 'tide comes in'.

A purpose of the foregoing remarks has been to show the desirability of possessing a simple and rugged means of determining the firmness of beaches (whether dry or water-covered up to neck depth for instance), in such fashion that investigators far removed from each other can record their findings in common terms. It has been thought that such a possibility might make some appeal to marine biologists as well as seeming obligatory upon students of beach and sea-bottom topography.

The device to be described was constructed for use with both instrument and user completely submerged in a connexion which need not concern us here, but, since the instrument is tall it can easily be used by an observer within all wading depths. In these days when some marine biological laboratories possess their own frogmen, wading depths need not be the limit—though of course, the greater the degree of immersion of the user, the less becomes his power to force any instrument into the sea bed beneath his feet. The tallness of the instrument was a deliberate choice to aid the user to effect a vertical approach to the ground being investigated.

In the case of bottoms carrying more than wading depth of water, unless probing from some firm platform like a jetty be possible, no acceptable means is known of recording firmness *in situ* other than by invoking the services of divers of frogmen type, and they often find it far from easy to operate corers and penetrometers specially made for them except when water movements are very weak.

DESCRIPTION OF THE PENETROMETER

For the accompanying drawing (Fig. 1) and the brief description which follows referring to it, I am indebted to Mr H. J. Garrood, A.M.I.M.E., who made the device to my instructions.

The penetrometer consists of a rod (1) pointed at the lower end (2). This rod is forced into the ground being investigated by pulling down by hand on the handles (6).

To the handles are attached two chains (7). These run over sprockets (8) and are fastened at their opposite end to a guide (9). The guide is connected through springs (10) to an anchor bracket (11). The guide (9) is able to slide on the rod (1).

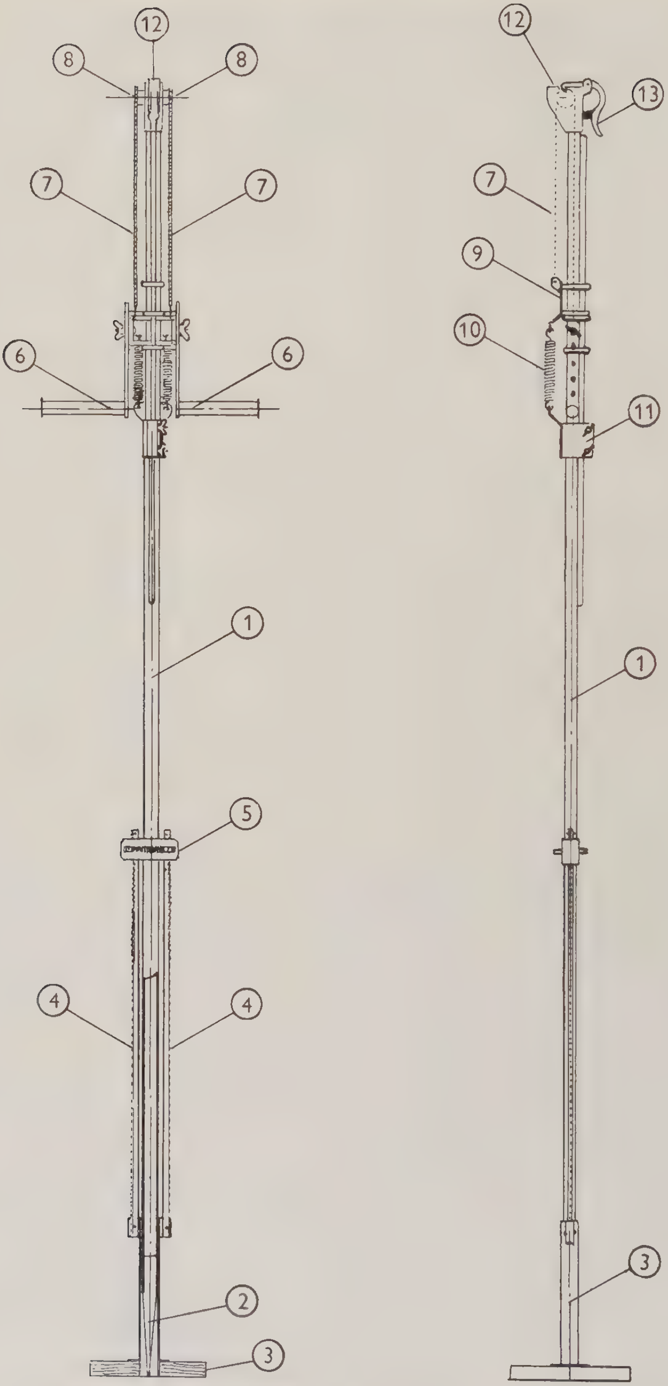
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Fig. 1. Diagram showing construction of the penetrometer. For explanation see text.

When the handles are pulled downwards, the rod penetrates the ground and the springs are stretched proportionately to the tension applied. A spring-loaded ratchet lever (13) engaging a toothed wheel (12) mounted between the chain sprockets and fixed to them, prevents the springs from relaxing when the pull is released. By measuring the stretch of the spring, the force which has been exerted on the rod may be ascertained.

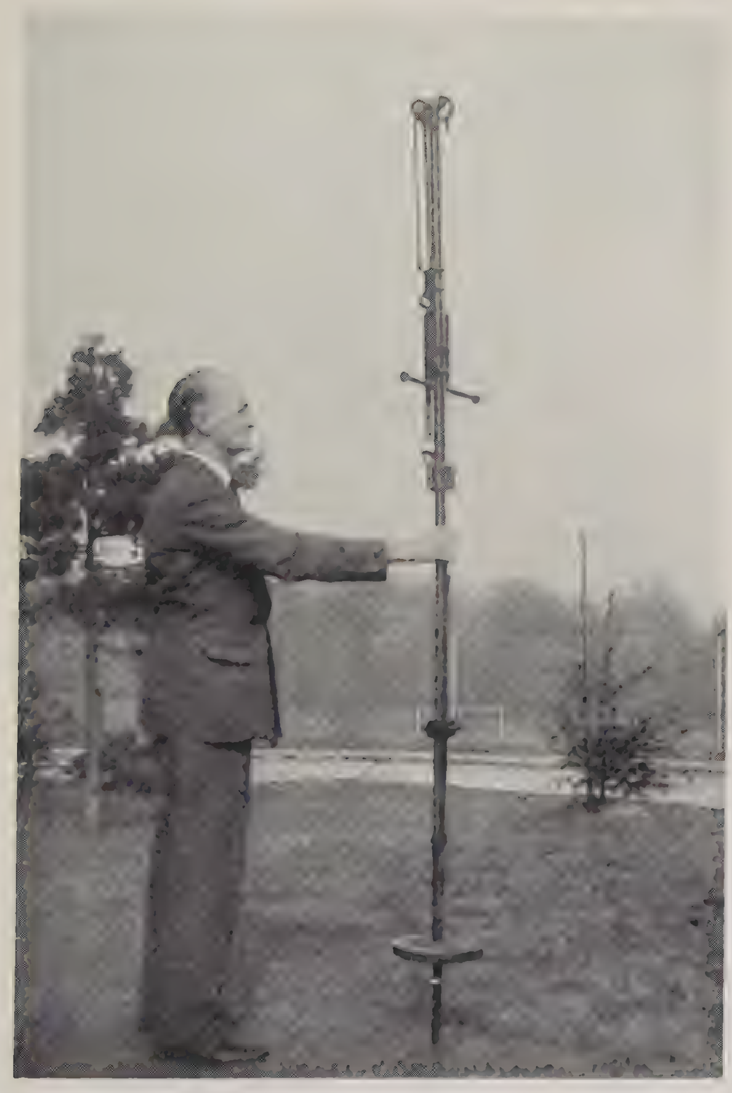

Fig. 2. Photograph of the submerged-beach penetrometer.

At the foot of the instrument a wooden disc attached to a tube (3) is arranged to slide on the rod. Moving with the disc and attached to it are two toothed racks (4) which pass through a bracket (5) fixed to the rod and having spring-loaded detents which engage in the ratchet teeth of the racks.

When the spear end of the rod penetrates the ground, the disc remains on the surface, but the bracket (5), moving with the rod, passes over the racks and is retained in its displaced position by the spring detents engaging in the teeth. The displacement of the disc relative to the point of the rod measures the depth of penetration. Comparison may thus be made between forces exerted and depths of penetration of various types of ground.

It will be appreciated that a stop can easily be affixed in order to always use a given tension.

The following are details as to size, weight and performance, and it should be remarked that exerted force can be very conveniently read off by 'measuring' the extended spring against a scale which is no more than a common white lath marked out to connect spring lengths with the weight loadings found in calibration tests to effect various extensions.

Overall height	265 cm
Total weight	11.3 kg
Handle length	15·2 cm
Rod circumference	9·1 cm
Rack length	71 cm
Disc diameter	22·9 cm
Spring length unstretched	30·5 cm
Spring length stretched:	
(*a*) by 14·7 kg weight	50·8 cm
(*b*) by 16·8 kg weight	58·4 cm
Penetration into loose recently dug soil	41·9 cm for 9·9 kg tension
Penetration into firm lawn	19·0 cm for 9·9 kg tension

N.B. The 'odd' dimensions and weights are due to conversion from British quantities into metric.

The accompanying photograph (Fig. 2) was kindly taken for me by my colleague Mr J. Darbyshire.

SUMMARY

Comments on the uses of penetrometers in general are followed by remarks upon an earlier one for use on dry beaches. Some discussion is given of the behaviour of beds of sand disturbed by shocks and converted thereby into a state of quasi-liquefaction. Finally, a description is given of a tall pull-down penetrometer which can be used on beaches covered by modest depths of water.

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J. Mar. biol. Ass. U.K. (1954) 33, 645–662
Printed in Great Britain

THE MIXING AND MOVEMENT OF WATER IN THE ESTUARY OF THE THAMES

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(Text-figs. 1–9)

In Great Britain many estuaries are seriously polluted by discharge of untreated or partially treated sewage and industrial waste waters. In addition to the unpleasant effects produced in the estuary itself, fisheries in the upper reaches may be damaged, as a barrier of deoxygenated or poisoned water may be formed through which migratory fish, such as salmon, are unable to pass. In some estuaries conditions are so bad that at times the water is completely deoxygenated; reduction of sulphate may then occur, resulting in the evolution of hydrogen sulphide which may cause nuisance from smell, blacken paint-work, and increase corrosion of metals.

When an effluent is discharged into a freshwater stream flowing in one direction only, the concentration in the water, at a given distance from the point of discharge, of any soluble component which decays at a known rate, can be calculated with reasonable accuracy. It is also possible to predict, although with greater error, the effect of oxidizable substances in the effluent on the dissolved oxygen content of the river water, provided the rate of re-oxygenation from the air is known (Phelps, 1947). It is much more difficult, however, to predict the effects of pollution in an estuary where the movement of water resulting from the inflow of fresh water and from tidal action is much more complicated. As estuaries are important economically the problem is being investigated in this country and in America (Ketchum, 1950, 1951).

In an estuary the inflow of fresh water tends to carry sea water seawards by displacement. On the other hand, sea water is carried towards the head of the estuary by the mixing brought about by tidal action. These two processes determine the salinity of the water in the estuary at any time, and the present paper suggests a method of calculating the degree of mixing in an estuary from observations of the flow of fresh water and the resultant salinities.

MIXING IN ESTUARIES

Types of estuaries

Stommel & Farmer (1952) divide estuaries into four types according to the degree of vertical mixing: (1) *Vertically mixed estuaries* are so thoroughly mixed vertically that there is no measurable difference in the salinity from the surface to the bottom. (2) *Slightly stratified estuaries* are characterized by only slight vertical stratification; the vertical mixing is intense. (3) *Highly stratified estuaries* show big differences of salinity between the surface and bottom; often there is no fresh water in the lower layer. In (4) *Salt-wedge estuaries*, the sea water intrudes up-stream as a wedge.

In this paper only estuaries of type 1 or 2 will be considered—and only those in which the salinity is sufficiently constant, both vertically and across the stream, to permit the intermingling of salt and fresh water to be regarded as a one-dimensional problem without appreciable error. An extension of the theory would be required for other types of estuaries.

The Thames Estuary (which is now being investigated by the Water Pollution Research Laboratory) is of type 2; not only the salinity but also the concentration of dissolved oxygen and other dissolved substances is almost constant in a cross-section. Pillsbury (1939) considered the relations between erosion of the bed and the tidal flow in estuaries which have little vertical salinity stratification. He showed that the width of such an estuary should increase exponentially towards the sea, and that the distance of tidal flow should be constant. The Thames is a good example of an estuary of this type. The width and the area of cross-section increase exponentially except in a region near Tilbury where the estuary is narrow and deep. The average length of tidal flow is everywhere about 8 or 9 miles except near Teddington Weir, and the mixing during one tidal period must be confined in a length of this order. The theory developed in this paper probably applies to all estuaries of the Pillsbury type.

Causes of mixing

The entry of water into an estuary from land sources displaces the water of the estuary towards the sea, the magnitude of the displacement at any point depending on the area of cross-section. If the estuary water moved without any mixing between adjacent sections, each unit of water pushing the unit in front of it without overtaking in any way, only the displacement and the oscillation due to the tide would occur, and the sea water would be driven from the estuary. As a result, however, of movement caused by both tidal and land-water flow, much mixing in fact takes place between the waters at different distances along the estuary. It may be expected that the dispersal of the water by mixing depends on many factors. One of these factors is the difference in the pattern of flow between the flood and ebb tides. If the area of cross-section is large, water which travels farthest up the estuary in the fastest currents may not mix completely at slack water, and the amount which is returned to the original neighbourhood will depend on the difference in position of the fastest currents of the ebb and flood tides. Turbulence, wind, waves, shipping, obstacles, and the nature of the bed will also influence the amount of mixing. At one place on the side of the Thames Estuary the flow of water is said to be always in one direction because of the formation of a very large eddy on the ebb tide; such eddies must also influence the amount of mixing. It is also to be expected that there will be a difference in the amount of dispersion of the water for spring and neap tides. In this paper the influence of the different tides will not be considered, but only the average amount of mixing during a long period will be calculated.

The movement of the water may be regarded as composed of three parts: displacement due to the flow from the land, tidal oscillation, and dispersal by mixing. Consider a unit of water which is contained between two cross-sections of the estuary which are very close to each other. Let the point, B, be midway between the cross-sections. At the corresponding time of the next tidal cycle this water will have been dispersed over the maximum distance of flow, to give a distribution curve similar to that shown in Fig. 1 *a*, in which R, the amount of the unit of water per unit of length, is plotted against distance along the estuary. The curve represents a dispersal which is additional to the displacement due to the entry of water from the land. R must be zero beyond the limits of the length of flow of the fastest currents during the tidal cycle, and is likely to be small beyond the mean distance of flow. Such a distribution curve exists for every point along the estuary. The water which has been dispersed during one tidal cycle will, during the next tide, be further dispersed according to the dispersion curves appropriate to the positions reached after the first tide. The result will be the dispersion curve corresponding to an interval of two tidal cycles; the sets of curves corresponding to different intervals of time can be calculated from each other.

The second moment of the curve is $\int_{-\infty}^{\infty} Rx^2\,\mathrm{d}x$, where x is the distance from the original position. It is well known that if a dispersal according to a distribution curve of second moment M_1 is followed by another dispersal of second moment M_2, the resulting distribution has a second moment $M_1 + M_2$. A set of dispersion curves exists for an estuary, and represents the mixing which causes sea water to move into the estuary against the general direction of flow. But if the water were repeatedly mixed in a way represented by a second set of dispersion curves, which differed in detail but had the same second moments, the movement would be almost identical. Hence it is not possible to calculate the exact shape of the curves from a consideration of the salinity balance during a long period. However, a complicated dispersion can be represented by a simpler dispersion which is repeated a number of times. For example, a binomial distribution approaches a normal distribution as the index increases. Hence a simplified form of the dispersion curves, with only a small number of arbitrary constants, can be taken, and the constants calculated, so that repeated mixing according to the simplified form is almost the same as the actual mixing in the estuary.

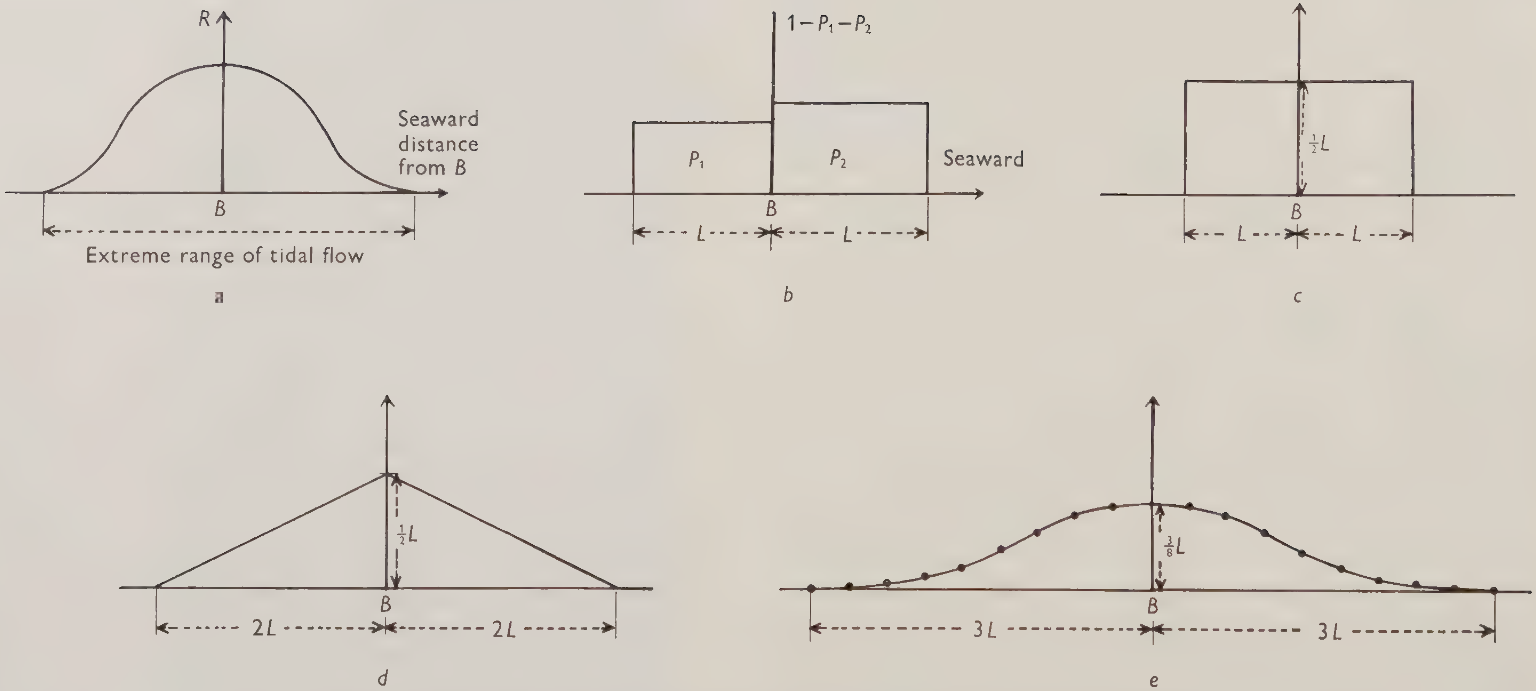
R
Seaward distance from B
B
Extreme range of tidal flow
a
$1-P_1-P_2$
P_1
P_2
Seaward
B
L
L
b
$\frac{1}{2}L$
B
L
L
c
$\frac{1}{2}L$
B
2L
2L
d
$\frac{3}{8}L$
B
3L
3L
e

Fig. 1. Diagrams illustrating distribution of a unit volume of water in an estuary. *a*, distribution caused by tidal mixing; *b*, assumed equivalent distribution. Successive dispersals when $P_1=P_2=\frac{1}{2}$; *c*, after time T; *d*, after time $2T$; *e*, after time $3T$.

Asymmetry of mixing

In an estuary of which the area of cross-section decreases in a direction away from the sea, finally dwindling into that of the river at the head, the dispersion curves, whether actual or the equivalent simplified form, cannot be symmetrical. The mixing causes some of the water from any particular section to move landwards into the adjacent, smaller, section, in which the salinity is lower. To make space for the more saline water, the water already in the upper section must move towards the sea by mixing. Hence it is necessary to take a form of the simplified dispersion curves which allows the centre of the water to move towards the sea. This need not be so in an inlet of constant area of cross-section, in which a unit of water might disperse symmetrically until it was unable to continue to do so because some of the water had reached the end of the inlet.

An example may make the equilibrium clear. Consider an estuary which can be divided into adjacent sections 1, 2, 3, ..., m of equal length, whose volumes are $v, 2v, 4v, \ldots, 2^{m-1}v$, respectively, the last section being next to the sea. The mixing can be considered as an exchange of water between the sections. If the amount exchanged is proportional to the volumes, and 1 unit is exchanged between the 1st and 2nd sections, then 2 units are exchanged between the 2nd and 3rd sections, 4 units between the 3rd and 4th sections, and so on, the last section exchanging 2^{m-1} units with the sea. The volume of water in each section remains constant, but the centre of gravity of the water of each section moves seaward.

REPRESENTATION OF THE MIXING

Since the detailed shape of the dispersal curves cannot be calculated from the salinity data, it is necessary to represent them in a simplified manner. It will be shown later that two conditions have to be satisfied at every point along the estuary, and hence each curve will have two arbitrary constants which can be determined from the measured salinities. As there are many families of curves which produce almost identical effects, if the water is dispersed many times, a method of representation which simplifies the mathematics may be selected. Consider a unit of water which is contained in a narrow cross-section of the estuary at B (Fig. 1*b*). After a lapse of time, T, the mixing may be represented as shown in the figure. P_1 is the proportion of the water which is distributed uniformly over a distance L towards the sea, and P_2 the proportion of the water distributed uniformly over the distance L in the opposite direction. The remainder of the water $1-P_1-P_2$ is left in its original position.

P_1 and P_2 have mathematical existence only. It is assumed that values exist for P_1, P_2 and L such that if the water is repeatedly mixed in accordance with these values, the dispersion of a unit of water is almost the same as the actual dispersion.

It is desirable that the assumed equivalent mixing should resemble the actual mixing in the estuary as closely as possible, so that agreement between the two systems of mixing occurs quickly and long calculations are avoided. This is the reason why $P_1 + P_2$ has not been equated to 1. A proportion, $1 - P_1 - P_2$, of the water is unmixed. If there is little mixing $P_1 + P_2$ is small and the proportion $1 - P_1 - P_2$ is large, corresponding to the large amount of water which must remain very near its original position in the estuary. It has already been shown that if T is equal to the tidal period, say T_0, then L should be of the same order as the mean length of flow of the tide. If $nT = T_0$, then L should be of the same order as the mean length of flow of the tide divided by $n^{\frac{1}{2}}$, by the theorem of addition of second moments. $P_1 + P_2$ must not be greater than 1. If the values of L taken lead to calculated values of $P_1 + P_2$ which are greater than unity in a section of the estuary, it follows that the mixing in this section is substantial over a greater distance than the assumed value of L. Hence L should be increased in the section, and the values of P_1 and P_2 recalculated. If at some point there is little mixing, then the actual dispersion curve for this point will have high values at its centre and low values at its extremes. This corresponds to low values of P_1 and P_2, and the assumed equivalent mixing curve also has high values at its centre, since the proportion of the water, $1 - P_1 - P_2$, which remains in its original position, is large. If the calculated values of P_1 and P_2 are exceedingly small, the actual mixing in the estuary can be substantial only in a shorter distance than L, and it is better to decrease L and recalculate P_1 and P_2.

An example of repeated mixing in accordance with numerical values of P_1 and P_2 may clarify the assumption which has been made. The simplest case is that when $P_1 = P_2 = \frac{1}{2}$ for all points in the estuary. The water, which was initially at the point B is, after an interval of time T, distributed as in Fig. 1*c*. During the next interval of time T, this water is further scattered from the position it has already reached, and its distribution becomes that of Fig. 1*d*. After a total lapse of time $3T$ the water has reached a position as shown in Fig. 1*e*, and the shape of its distribution curve is approaching that of a normal error curve, as, of course, it must.

Equations relating P_1 and P_2 to observable data

The two following conditions must be satisfied. (i) During any period the net amount of salt carried upstream past any point is equal to the amount present above this point at the end of the period, *minus* the amount present at the beginning, *minus* any amount discharged into the estuary above the point during the period considered. (ii) The volume of water carried upstream past the point during the period must conform to a similar condition.

The volume of fresh water entering the estuary is completely accounted for by the displacement mentioned above, and hence the values of P_1, P_2 and

L must be such that the mixing process alone does not cause a net flow of water either upstream or downstream.

The salinity data available for the Thames Estuary made it convenient to consider conditions at half tide, which is defined as the time at which the height of the water is the mean of the heights at high and low tide.

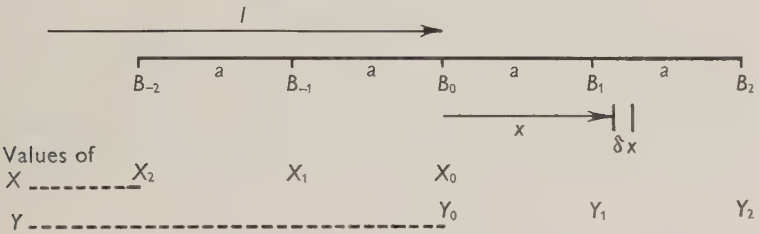

Fig. 2. Diagram of symbols used in calculation by relaxation.

Consider a point, B_0, at a distance l along the estuary (Fig. 2), and let A be the average area of cross-section of the river at half-tide. During a tidal cycle, salt in the water in the neighbourhood of B_0 is caused to flow past B_0 by the mixing. From a section between distances of x and $x+\delta x$ from B_0, the amount of salt which is carried past B_0 by the mixing during time T is

$$\frac{L-x}{L}CAP_2\delta x,$$

where C is the concentration of salt in the water, and the amount of salt which flows past B_0 during n intervals of time T is

$$\sum_1^n CAP_2\frac{L-x}{L}\delta x.$$

The values of C, A, P_2 and L are, of course, those at the point $1+x$.

The net amount of salt which is carried upstream past B_0 during the n intervals of time, T, is

$$\int_0^L\sum_1^n CAP_2\frac{L-x}{L}\mathrm{d}x+\int_0^{-L}\sum_1^n CAP_1\frac{L+x}{L}\mathrm{d}x. \qquad (1)$$

The amount carried seaward by the flow of water along the estuary is $\sum_1^n FC$, where F is the net flow of land water during 1 interval of time, T. From condition (i)

$$\frac{1}{n}\left[\int_0^L\sum_1^n CAP_2\frac{L-x}{L}\mathrm{d}x+\int_0^{-L}\sum_1^n CAP_1\frac{L+x}{L}\mathrm{d}x\right]$$
$$=\frac{1}{n}\left[\sum_1^n FC+S_2-S_1\right], \qquad (2)$$

where S_2 and S_1 are the amounts of salt above B_0 at the end and the beginning of the period respectively. Or

$$\int_0^L \overline{C}Y\frac{L-x}{L}\,dx+\int_0^{-L}\overline{C}X\frac{L+x}{L}\,dx=\overline{FC}+S, \tag{3}$$

where $\overline{C}=\frac{1}{n}\sum_1^n C=$ the average salt concentration,

$X=AP_1$, $Y=AP_2$,

$\overline{FC}=\sum_1^n FC/n=$ the average product of salt concentration and flow,

$S=\dfrac{S_2-S_1}{n}$.

Similarly, from condition (ii)

$$\int_0^L Y\frac{L-x}{L}\,dx+\int_0^{-L}X\frac{L+x}{L}\,dx=0. \tag{4}$$

From records of the salinities and of the flows of fresh water into the estuary, the numerical values of $\overline{C}$ and $\overline{FC}$ can be computed for the places of sampling and can be obtained at intermediate points by interpolation. The period for which the means are found should be long enough to give average conditions. The individual readings are not required to obtain X and Y, which are calculated from equations (3) and (4); only the averages $\overline{C}$ and $\overline{FC}$ are necessary. The values of $\overline{C}$ for the Thames Estuary from 1 January to 18 December are shown in Fig. 3. This graph, and a similar graph of $\overline{FC}$, provided the only experimental data of salinities which were used in the calculation of the values of X and Y for the Thames Estuary.

Solution by relaxation

Equations (3) and (4) have to be solved for X and Y numerically. This can be done by the relaxation method.

Let $B_{-2}, B_{-1}, B_0, B_1, B_2$ be points along the estuary, each separated by the distance a (Fig. 2), let X_2, X_1, X_0 be the values of X at B_{-2}, B_{-1}, B_0 respectively, and let Y_0, Y_1, Y_2 be the values of Y at B_0, B_1, B_2, respectively.

As a first approximation, X, Y and $\overline{C}$ may be considered as linear functions of distance within the range of the mixing length L and

$$X=X_0-(X_1-X_0)x/a \quad \text{from } B_0 \text{ to } B_{-1},$$

$$Y=Y_0+(Y_1-Y_0)x/a \quad \text{from } B_0 \text{ to } B_1,$$

$$\overline{C}=C_0+C_1x \quad \text{from } B_{-1} \text{ to } B_1,$$

where C_0 and C_1 are constants, and x is the distance from B_0.

Substitution in equations (3) and (4), and simplification, gives

$$2X_0L^2+2Y_0L^2+\frac{(Y_1-Y_0)L^3}{a}+\frac{(X_1-X_0)L^3}{a}=\frac{12Q}{C_1}, \tag{5}$$

and

$$3Y_0L-3X_0L+\frac{(Y_1-Y_0)L^2}{a}-\frac{(X_1-X_0)L^2}{a}=0, \tag{6}$$

where $Q=\overline{FC}+S$.

Equations (5) and (6) exist for all the points in the estuary, and can be used to obtain the approximate values of X and Y at a series of points by the relaxation method. If $a=L$, equations (5) and (6) become

$$X_0+Y_0+Y_1+X_1=12Q/L^2C_1, \tag{7}$$

$$2Y_0-2X_0+Y_1-X_1=0. \tag{8}$$

To obtain greater accuracy it is necessary to take $\overline{C}$, X and Y as quadratic functions of distance in the range of the mixing lengths and put

$$X=X_0-\frac{(4X_1-3X_0-X_2)x}{2a}+\frac{(X_2+X_0-2X_1)x^2}{2a^2},$$

$$Y=Y_0+\frac{(4Y_1-3Y_0-Y_2)x}{2a}+\frac{(Y_2+Y_0-2Y_1)x^2}{2a^2},$$

$$\overline{C}=C_0+C_1x+C_2x^2,$$

where C_0, C_1 and C_2 are constants. On substitution, equations (3) and (4) become

$$X_0[10C_1L^2-5C_2L^3]+Y_0[10C_1L^2+5C_2L^3]+[4Y_1-3Y_0-Y_2]\,[5C_1L^3+3C_2L^4]/2a+[4X_1-3X_0-X_2]\,[5C_1L^3-3C_2L^4]/2a+[X_2+X_0-2X_1]\times[3C_1L^4-2C_2L^5]/2a^2+[Y_2+Y_0-2Y_1]\,[3C_1L^4+2C_2L^5]/2a^2=60Q, \tag{9}$$

and

$$6X_0L-6Y_0L+(4X_1-3X_0-X_2)L^2/a-(4Y_1-3Y_0-Y_2)L^2/a+(X_2+X_0-2X_1)L^3/2a^2-(Y_2+Y_0-2Y_1)L^3/2a^2=0. \tag{10}$$

If $2a=L$, equations (9) and (10) become

$$C_1[X_0+Y_0+8X_1+8Y_1+X_2+Y_2]+LC_2[4Y_1-4X_1+Y_2-X_2]=60Q/L^2 \tag{11}$$

and

$$2X_1-2Y_1+X_0-Y_0=0. \tag{12}$$

These equations can be used to improve the values obtained from the linear equations. It is advisable to obtain the first approximate solution for a set of points whose distances apart are L and then to introduce the values of X and Y at points midway between the first points when the quadratic equations are used. Group relaxation greatly shortens the work. Decreasing the distance between the points does not produce greater accuracy, unless the values at all the points within the range of the integral are used to give more accurate relaxation formulae. If $3a \leqslant L$, cubic or higher equations have to be used, and the relaxation becomes very cumbersome and difficult. If greater accuracy than that obtainable by the quadratic equations is required, it is better to use numerical integration or some other method. A little experience will show how to diminish the remaining error when this is found by numerical integration.

Any values of X and Y may be assumed at the start of the relaxation, but time will be saved if these values are reasonably accurate. For the period selected for the Thames Estuary it was found that for a long distance both $\overline{C}$ and Q were approximately linear functions of distance given by

$$\overline{C}=\theta_1(l+2), \tag{13}$$

$$Q=\theta_2(l+2), \tag{14}$$

where θ_1 and θ_2 are constants, and l is the distance in miles from London Bridge. Substitution in equations (3) and (4) shows that the following equations are good approximate solutions:

$$X=\frac{3l+6+L}{L^2}\,\frac{\theta_2}{\theta_1}, \tag{15}$$

$$Y=\frac{3l+6-L}{L^2}\,\frac{\theta_2}{\theta_1}. \tag{16}$$

The values given by equations (15) and (16) were used as the initial values in the relaxation for the Thames Estuary.

SOURCES OF INFORMATION FOR THE THAMES ESTUARY

Salinity

The London County Council has a very extensive record of the salinities of the Thames Estuary from London Bridge to the Nore, from 1920 to the present day. Salinities of samples taken at a depth of 6 ft. in mid-stream are recorded weekly at many sampling positions. Times of sampling vary, but most of the samples from any one position have been taken at about the same state of the tide. The London County Council very kindly allowed full use to be made of these records.

Tides

The Port of London Authority has supplied much information regarding the tides, including the results of simultaneous tidal surveys at many points in the estuary. Data from the Admiralty Tide Tables have also been used.

Flow

The Thames Conservancy records the daily flow over Teddington Weir. Low flows are measured with accuracy, but there may be greater error in the high winter flows. Figures for the flow of tributaries and effluents have been provided by River Boards, Local Authorities, and industries. Much information has also been obtained from the survey of the estuary now being made.

Correction of salinity position

The readings of the L.C.C. cannot be used directly to find the salinity gradient along the estuary, as samples from different points are taken at different states of the tide. The fact that there is little salinity variation in a cross-section of the estuary, suggests that when the salinities of the estuary are in equilibrium, water of a constant salinity moves with the tide so that the volume of water between it and Teddington Weir is constant. If this is so, then the position of a sample can be adjusted to a half-tide position so that the volume of water between the sample and Teddington Weir remains the same. This has been done for a great number of observations and it has been found that salinities and concentrations of chemicals dissolved in the water then fall on a smooth curve. The correction may be large compared with the distance between the sampling points. Fig. 3 shows a corrected curve for the average values of the salinities from 1 January to 18 December 1948 plotted against distance along the estuary.

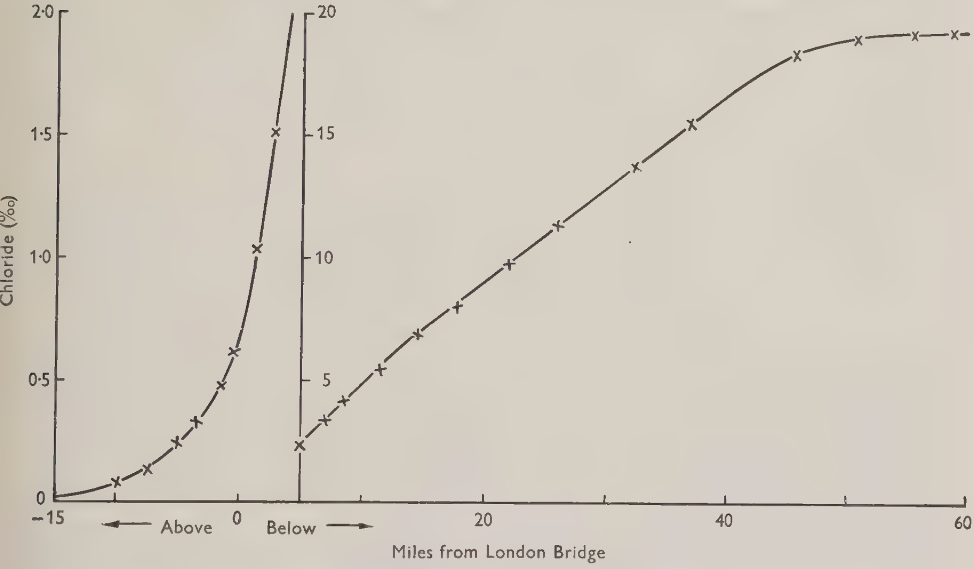

Fig. 3. Average contents of chloride in the Thames Estuary, 1 January to 18 December 1948. Chloride given as parts per thousand.

RESULTS FOR THE THAMES ESTUARY

In calculating the extent of mixing in the Thames estuary, L was given the value 4·5 miles for mixing during half of the average tidal period, i.e. for $T=6$ h $12\frac{1}{2}$ min. For $T=24$ h 50 min, the time of two tides, L was given the value of 9 miles. The calculation was made from the average values of salt concentration, and the average values of the product of salt concentration and volume of land water entering the landward section of the estuary, from 1 January to 18 December 1948. A longer period could not be used because of lack of information regarding the flows of the tributaries of the estuary at other times. Above London Bridge the average value of salt concentration became small, and in the section of the estuary near to Teddington (19 miles above London Bridge) X and Y could not be obtained accurately because here the salt contents of effluents (mainly sewage effluents) and of the upper river were important, and were not known with sufficient accuracy. Fortunately, in this part of the estuary, the flow of land water is dominant, and it may be expected that the theory of the effects of pollution developed for rivers flowing in one direction only can be applied.

The values of X and Y which were obtained (and which correspond to a time interval of two tidal periods) are shown in Fig. 4. The corresponding values of P_1, P_2 and $1-P_1-P_2$ below London Bridge are shown in Fig. 5. X and Y increase steadily towards the sea and X is everywhere greater than the corresponding value for Y. P_1 and P_2 show irregularities at places where the area of cross-section is irregular. The area of cross-section is approximately an exponential function of distance along the estuary, as required by Pillsbury's theory for this type of estuary. Where the area is greater than that given by Pillsbury's relation, P_1 and P_2 are less, though X and Y remain uniform. This is to be expected since if, for example, the estuary widened to form a bay, the water in the bay would be likely to remain relatively undisturbed during the tide making P_1 and P_2 small in that region, but not altering X and Y. P_1+P_2 is a measure of the amount of dispersion of a unit of water, and this varies along the estuary, being a maximum 25 miles below London Bridge.

X, Y, P_1 and P_2 probably vary with different ranges of tide. The values obtained are averages for the tides of the period considered.

Verification

If the salinities at a given time and the flows of fresh water during the following period are known, the salinity at the end of the period may be predicted from a knowledge of X and Y obtained from equations (3) and (4). The period must be divided into intervals of time for which the mixing values have been found, and the salinity at the end of each successive interval can be found by: (1) displacing the water to allow for the natural flow, and then (2) calculating the new salinities at a number of points by numerical integration of the expression

$$\frac{1}{A}\left[\int_0^L \frac{CY}{L}\,dx+\int_{-L}^0 \frac{CX}{L}\,dx+C(A-X-Y)\right], \tag{17}$$

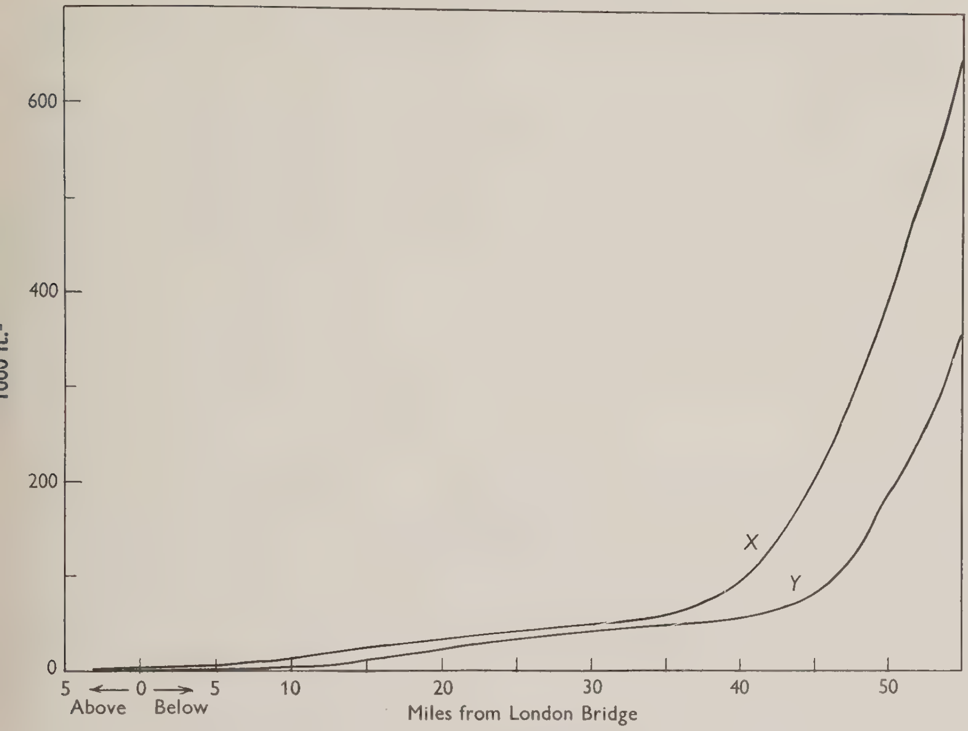

Fig. 4. Values for X and Y when T equals two tidal periods.

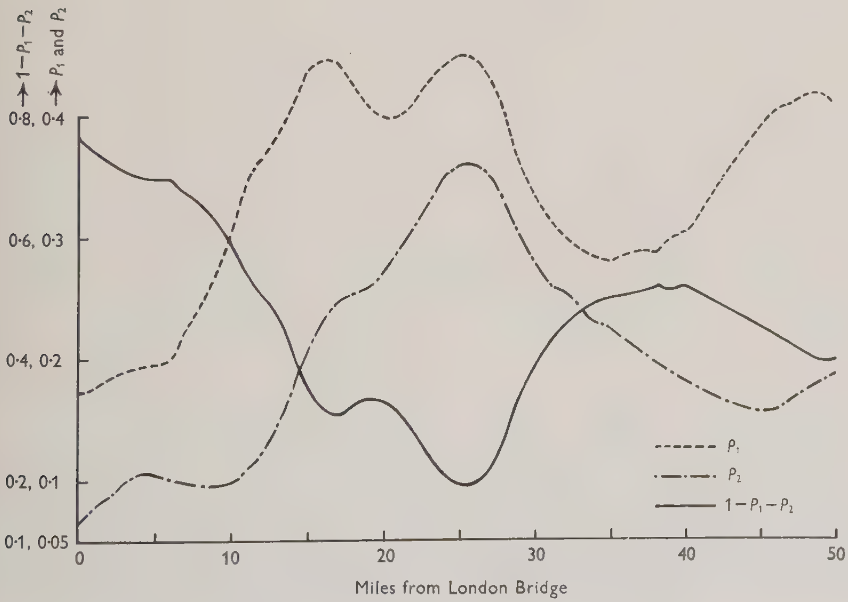

Fig. 5. Values of P_1 and P_2 for a period of two tides.

which gives the new concentration at the point which results from the mixing of the water in the neighbourhood. x is the distance from the point at which the salinity is being calculated.

The expression (17) can be derived as follows. Let the point where the new concentration of salt is to be calculated be B, and let x be the distance from this point. A proportion $1-P_1-P_2$ of the salt at B remains there and makes a contribution $(1-P_1-P_2)\,C$ to the new concentration at B. From the salt in the range x to $x+\delta x$, a proportion P_2 is carried towards B by the mixing if $0<x\leqslant L$. This contributes $\dfrac{CP_2A}{AL}\,\delta x$ to the new concentration at B, and the total contribution from the range $0<x\leqslant L$ is

$$\int_0^L \frac{CP_2A}{AL}\,dx=\int_0^L \frac{CY}{AL}\,\mathrm{d}x.$$

Similarly, the contribution from the range $0>x\geqslant -L$ is

$$\int_{-L}^{0} \frac{CX}{AL}\,\mathrm{d}x,$$

and the new concentration of salt at B is

$$\frac{1}{A}\left[\int_0^L \frac{CY}{L}\,\mathrm{d}x+\int_{-L}^{0}\frac{CX}{L}\,\mathrm{d}x+C(A-X-Y)\right].$$

Figs. 6 and 7 show the results of two calculations. Curve 1 of both figures is obtained from salinity measurements made by the L.C.C. In the first the flow of river water over Teddington Weir increased during the following 14 days, and in the second it decreased. From the displacements of the estuary water corresponding to the flows and the values of the mixing constants, the expected salinity changes were calculated. The second curve shows the calculated result after an interval of 14 days; intermediate stages are not shown. The corresponding salinities observed by the L.C.C. at about the same time are also shown. The L.C.C. does not take samples from all parts of the estuary at the same time, and some of the corresponding salinity values were taken at slightly different times. This accounts for some of the discrepancy between the observed and predicted curves. Also the flows over Teddington Weir were high—a condition where the maximum error in measurement is to be expected.

It is possible to obtain the salinities which are in equilibrium with a given constant flow of water from land sources, by repeatedly calculating the salinity changes which would occur with this flow, until a salinity distribution which shows no further change is obtained. The salt concentrations calculated to be in equilibrium with the average flow during 1946 (the flows of minor tributaries were not known accurately) are shown in Fig. 8 with the average of the

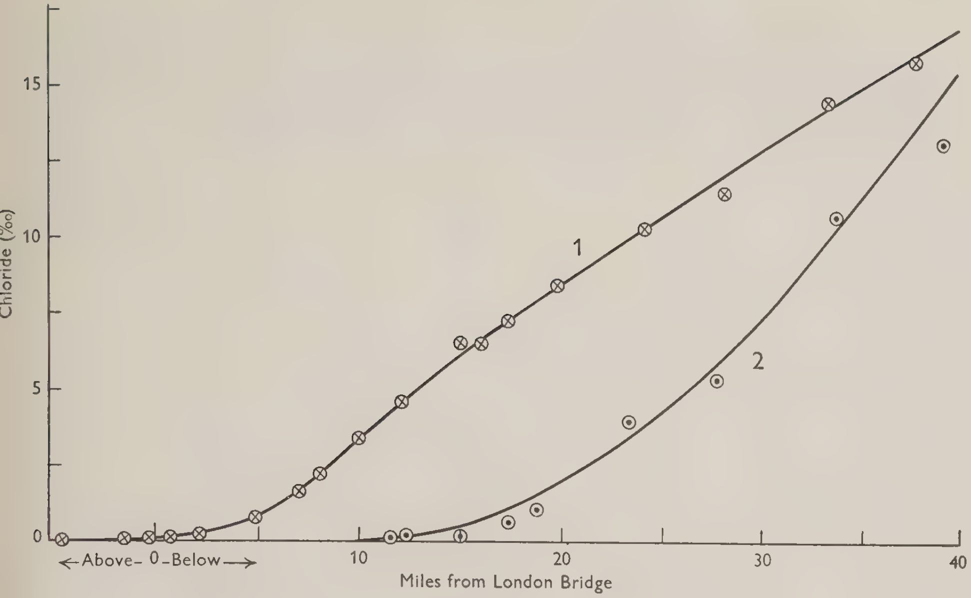

Fig. 6.

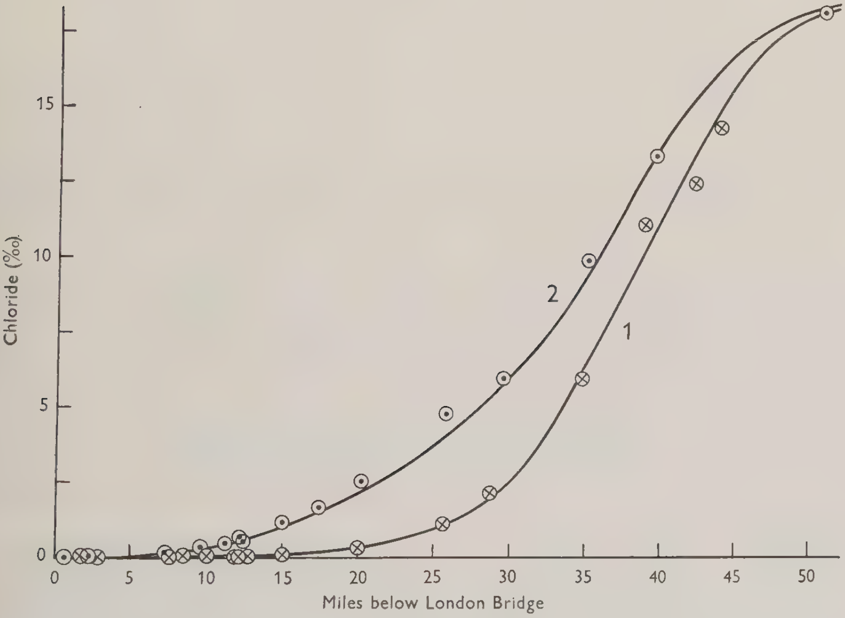

Fig. 7.

Figs. 6 and 7. Curves showing accuracy of calculation of changes in salinity in the Thames Estuary. Curve 1 was plotted through the readings ⊗ taken on a zero date. Curve 2 was calculated from curve 1, being an estimate for the conditions 14 days later. The readings ⊙ were the observed values on that day.

salt concentrations found by the L.C.C. during the year. The agreement is surprisingly good since some difference is expected. At some point in the freshwater end of the estuary the concentration of salt which is in equilibrium with the average annual flow approaches zero. Near this point, the observed average salinities must be greater than the equilibrium salinity since at times when the flow of fresh water is very small the salinity increases appreciably, while at times when the flow of fresh water is large the salinity cannot be much less than the calculated salinity since this is small.

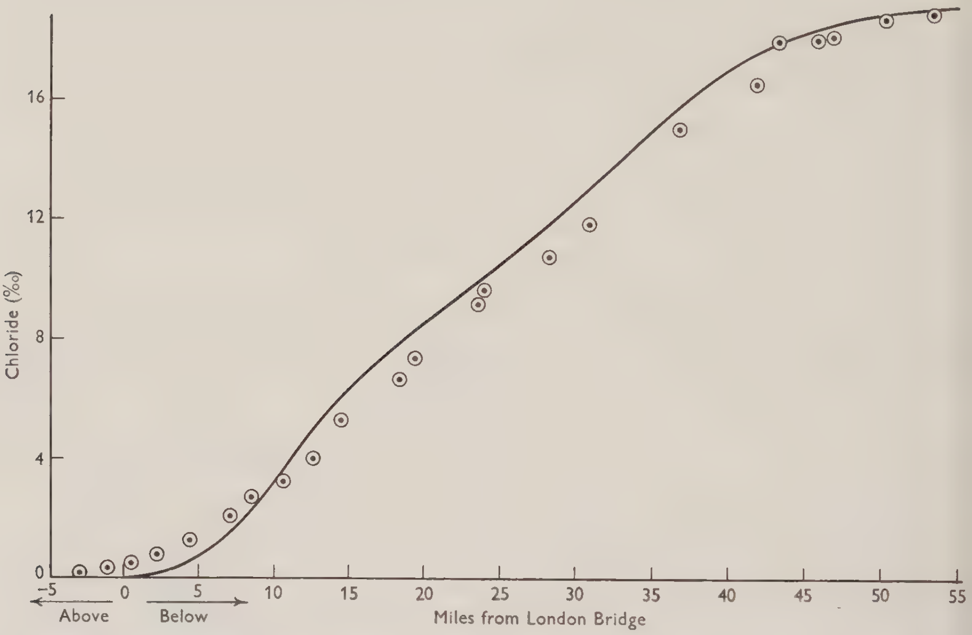

Fig. 8. Average content of chloride in the Thames Estuary, 1946. The continuous line plots values calculated to be in equilibrium with the mean flow of fresh water during the year. The circles represent means of L.C.C. observations during the year.

Application to Pollution Problems

The application of the theory to problems of pollution will be considered only very briefly.

If there is tidal flow past a point of discharge of an effluent, the effluent is distributed at once throughout the water which flows past the point. This distribution (which is, of course, not uniform) can be calculated from the tidal data and is equivalent to a discharge over a length $2L$ at half-tide. Hence the initial concentration of the effluent in the water can be found and the concentration at any point at a later time can be calculated by the process already given for salinities. Fig. 9 shows the calculated distributions along the Thames Estuary of the effluent discharged from the Northern Outfall of the L.C.C. when the flow of fresh water over Teddington Weir is 500 million gallons per day. The extent of pollution of the water, as measured by the biochemical oxygen demand, can be calculated from these curves if the rate at which the demand is satisfied is known. If the rate of solution of oxygen from the air is also known, it is then possible to calculate the level of oxygenation of the water, using the mixing constants. For the Thames Estuary, where there are a great number of discharges, the calculation by this method is very tedious, but it seems likely that methods can be developed which will simplify the arithmetical work involved.

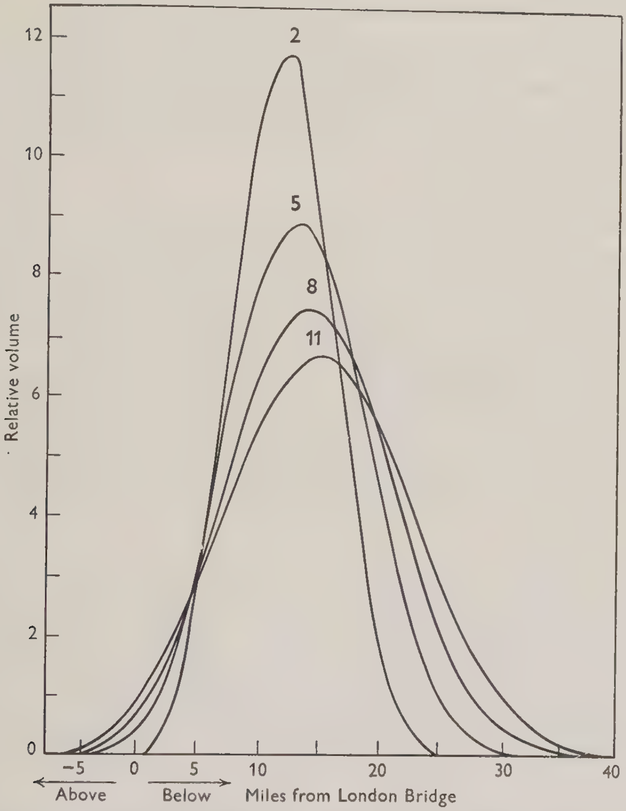

Fig. 9. Computed distribution of a liquid discharged into the Thames Estuary, during a period between low and high water, at the Northern Outfall of the London County Council sewage-disposal works. The number of tidal cycles after discharge is shown against each curve. The vertical scale shows the comparative volume of added liquids per unit length of river. Flow of fresh water at Teddington = 500 m.g.d.

Much of the numerical calculation for this work was done by Mr L. J. Jeeves, who also collected many of the data, and made many useful suggestions. I should like to thank Mr C. J. Regan, Chief Chemist of the L.C.C., for many valuable discussions.

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SUMMARY

A method of representing the mixing of water in an estuary is given, and the amount of mixing which occurs in the Thames Estuary is calculated. Changes in salinity predicted from the results are found to agree with observation. The application of this theory of mixing to some pollution problems is briefly considered.

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J Mar. biol. Ass. U.K. (1954) 33, 663–676
Printed in Great Britain

THE OCCURRENCE OF PORPHYRINS IN CERTAIN MARINE INVERTEBRATES

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(Text-fig. 1)

Kennedy & Vevers (1953 *a*, *b*) confirmed the presence of a porphyrin in the integument of the starfish *Asterias rubens*, reported by MacMunn (1887), and showed that it was protoporphyrin. A yield of 33·6 mg of protoporphyrin dimethyl ester was obtained from 548·5 g of *Asterias* integument. Following this work, and the investigation of porphyrins in mollusc shells by Nicholas & Comfort (1949), it was naturally of interest to examine the distribution of porphyrin pigments in the soft and skeletal parts of other marine animals. A survey has therefore been made of the occurrence of porphyrins in certain invertebrates common in the waters off Plymouth.

METHODS

The animals were examined first in the fresh state under ultra-violet light (Osira 125 W black glass lamp) and then homogenised (in an Atomix blender) and extracted by the methods used previously (Kennedy & Vevers, 1953 *b*). The extracts in turn were examined by ultra-violet light and the occurrence of red fluorescence noted. Where extracts showed red fluorescence the source of this was investigated chemically and, where possible, the pigments responsible for this fluorescence were isolated and characterized by the following methods:

(1) *Solubility* in ether, chloroform, ethyl acetate, 0·1N hydrochloric acid, and 0·1N sodium hydroxide.
(2) *Partition* between ether and increasing concentrations of aqueous hydrochloric acid from 0·1% (w/v), this is the HCl number (Salzsäurezahl) of Willstätter & Stoll (1913).
(3) *Column chromatography*, Nicholas (1951) and Kennedy (1953 *a*).
(4) *Paper partition chromatography* Nicholas & Rimington (1949), Kennedy (1953 *b*) and Chu, Green & Chu (1951).
(5) *Absorption spectra*, using a Beck-Hartridge Reversion spectroscope, and a Unicam S.P. 500 spectrophotometer.
(6) *Formation of derivatives* and conversion to other porphyrins, when sufficient material was available.

TABLE I. OCCURRENCE OF RED FLUORESCENCE IN EXTRACTS FROM CERTAIN MARINE INVERTEBRATES

(Extracts of whole animal except where otherwise stated.)

	Red fluorescence due to		
	Porphyrins	Chlorophyll and derivatives	Remarks
PORIFERA			
Sycon coronatum	—	—	.
Grantia compressa	—	—	.
Tethya aurantium	?+	—	Not yet identified
Halichondria panicea	—	+	Algae
COELENTERATA			
Velella spirans (skeleton)	—	—	.
V. spirans (soft parts)	—	—	.
Alcyonium digitatum	—	—	.
Actinia equina	—	—	.
Anemonia sulcata	—	+	Algal symbionts
Tealia felina	—	—	.
Metridium senile	—	—	.
Calliactis parasitica	—	—	.
ANNELIDA			
Aphrodite aculeata	—	—	.
Nereis diversicolor	+	+(*a*)	.
Perinereis cultrifera	—	—	.
Chaetopterus variopedatus	+	+(*b*)	.
Arenicola marina	—	—	.
Myxicola infundibulum	—	—	.
M. infundibulum (purple tentacles)	—	—	.
M. infundibulum (alimentary system)	+	—	.
ARTHROPODA			
Portunus depurator	—	—	.
Carcinides moenas (soft parts)	—	+	From diet in alimentary tract
C. moenas (carapace)	—	—	.
Cancer pagurus (eggs)	—	—	.
C. pagurus (viscera)	—	—	.
C. pagurus (claw shell)	—	—	.
C. pagurus (carapace)	—	—	.
Eupagurus bernhardus (soft parts)	—	—	.
MOLLUSCA			
Mytilus edulis	—	—	.
M. edulis (shell)	—	—	.
Chlamys opercularis	—	—	.
C. opercularis (mantle)	—	—	.
Cardium edule (soft parts)	—	—	.
Buccinum undatum (soft parts)	—	—	.
Scaphander lignarius (bursae)	—	—	.
Philine aperta (bursae)	—	—	.
Aplysia punctata (integument)	+	—	.
Jorunna tomentosa	—	—	.
Archidoris britannica	—	—	.
A. britannica (eggs)	—	+	Chlorophyll from Algae
Duvaucelia plebeia (upper integument)	+	—	.
Loligo forbesi	—	—	.
Parasepia elegans	—	—	.

TABLE I (*continued*)

	Red fluorescence due to		
	Porphyrins	Chlorophyll and derivatives	Remarks
ECHINODERMATA			
Antedon bifida	–	–	.
Astropecten irregularis (integument)	+	–	.
Luidia ciliaris (integument)	+	–	.
Porania pulvillus (integument)	–	–	.
Palmipes membranaceus (integument)	–	–	.
Solaster papposus (integument)	–	–	.
Henricia sanguinolenta (integument)	–	–	.
Asterias rubens (integument)	+	–	.
Marthasterias glacialis (integument)	–	–	.
M. glacialis (spicules)	–	–	.
Ophiothrix fragilis	–	–	.
Ophiocomina nigra	–	–	.
Psammechinus miliaris (test)	–	+	Chlorophyll from Algae on test
Holothuria forskali (integument)	–	–	.
TUNICATA			
Ascidiella aspersa (viscera)	–	–	.
Ciona intestinalis (viscera)	–	–	.

(*a*) Phaeophorbide *a*; (*b*) Phaeophorbides *a* and *b*.

The results of this survey are given in Table I, and further details of those animals in which porphyrins were found are given in Table II. Where positive identification of pigments was possible the evidence upon which this was based is described under the name of the pigment.

TABLE II. OCCURRENCE OF PORPHYRINS IN CERTAIN MARINE INVERTEBRATES

Phylum	Species	Porphyrin	Distribution
Annelida	*Nereis diversicolor*	Coproporphyrin III	Viscera
	Chaetopterus variopedatus	Coproporphyrin III and a pentacarboxylic porphyrin	Viscera
	Myxicola infundibulum	Coproporphyrin III	Viscera
Mollusca	*Aplysia punctata*	Uroporphyrin I	Integument
	Duvaucelia plebeia	Uroporphyrin I	Integument
Echinodermata	*Astropecten irregularis*	Chlorocruoroporphyrin and protoporphyrin	Integument
	Luidia ciliaris	Chlorocruoroporphyrin and protoporphyrin	Integument
	Asterias rubens	Protoporphyrin	Integument

IDENTIFICATION OF PIGMENTS

Coproporphyrin III

This pigment was noticed during the course of paper partition chromatography (Nicholas & Rimington, 1949) of chloroform extracts of the three polychaetes—*Nereis*, *Chaetopterus* and *Myxicola*—in an investigation of the green pigment 'chaetopterin' (with J. A. C. Nicol), to be reported in the near future. The long-paper method of Kennedy (1953*b*) was employed, with 2:6-lutidine and water as solvent phases. A well defined spot appeared, with strong red fluorescence at R_F 0·65, which suggested a 4-COOH porphyrin, and coproporphyrin in particular. Further chromatograms were run under the same conditions, but including on each paper a spot of coproporphyrin I as a marker, in both adjacent and mixed spots. Only one spot appeared at R_F 0·65 on the mixed spot papers, and on the papers with adjacent spots there were two spots side by side at R_F 0·65. This confirmed the presence of a 4-COOH porphyrin, and strongly suggested coproporphyrin.

There was very little material for experiment so the spots of all the long-paper chromatography experiments were cut out carefully, the pieces soaked in a little dry pyridine and examined spectrophotometrically. The spectrum showed the maxima:

I	II	III	IV
623·6	569·0	533·0	499·0 mμ

further indicating that this porphyrin is indeed a coproporphyrin.

Concentrated solutions of the extracts from the three worms were then evaporated to dryness *in vacuo*, the residues esterified with methanol/hydrochloric acid and examined by the double-development paper-chromatography method of Chu *et al.* (1951), using chloroform: kerosene/*n*-propanol: kerosene at 19° C. Well-marked spots were observed with centre-dense red fluorescence at R_F 0·81, corresponding to coproporphyrin III. The test was repeated with markers of coproporphyrin I tetramethyl ester and coproporphyrin III tetramethyl ester (the latter very kindly supplied by Prof. C. Rimington, F.R.S.) in both adjacent and mixed spots. On those papers bearing the extracts and coproporphyrin I in mixed spots, two clear sets of spots were seen, indicating that the two porphyrins were not identical. On those papers bearing adjacent spots of coproporphyrin I and extract, there was a wide difference between the R_F values.

Papers carrying mixed spots of the worm extracts with authentic coproporphyrin III showed only one set of spots at R_F 0·80 confirming that the worm porphyrin was, in fact, identical with coproporphyrin III. Papers bearing adjacent spots of the coproporphyrin III ester and the worm extract showed that both pigments travelled together in parallel, and took up the same R_F position, 0·80. This established that the worm porphyrin was present as coproporphyrin III.

A Penta-carboxylic Porphyrin

In the course of these long-paper chromatography experiments just described, extracts of *Chaetopterus variopedatus* produced a spot above that of coproporphyrin with an R_F value of 0·5. This is indicative of a porphyrin with five carboxyl groups. This was repeated several times and confirmed, but there was insufficient material for exact characterization. Further material is being collected for a detailed examination of this porphyrin.

Uroporphyrin

The upper integument of the nudibranch gastropod *Duvaucelia* (*Tritonia*) *plebeia* yielded no red fluorescent material to ether/acetic acid, but on placing the tissue in a mixture of absolute methanol (19 parts) and concentrated sulphuric acid (1 part) overnight the extract was found to be slightly red fluorescent to ultra-violet light. This fluorescence increased very much in intensity on diluting the extract with water, shaking up with chloroform and then examining the chloroform hypophase under ultra-violet light.

The chloroform extract was roughly dried by filtration and evaporated to dryness, and the residue was then re-dissolved in chloroform. This chloroform extract was passed through a column of alumina grade IV (Nicholas, 1951), packed by sprinkling into chloroform, and a broad red fluorescent band formed at the top of the column, with a diffuse pinkish non-fluorescent band below. The top band was immovable with ethanol/chloroform mixtures, ether, ether/pyridine mixtures, acetone or ethyl acetate, but was eventually eluted with the methanol/sulphuric acid (19:1) esterification mixture. The pigment was collected as a purple-red solution, intensely red fluorescent. A greyish non-fluorescent band was left on the column.

The acid/methanol solution was diluted with two-thirds of its volume of water, and the pigment was extracted with chloroform to form a purple hypophase with intense red fluorescence. The hypophase was then separated, washed several times with 2% sodium chloride, and finally with water. The solution was roughly dried by filtration through chloroform-soaked paper and evaporated to dryness on the water-bath. The pigment was redissolved in dry chloroform and the absorption spectrum determined spectrophotometrically (Unicam S.P. 500), and plotted as $E_{1\text{ cm}}$ against wave-length. The maxima were:

I	II	III	IV	Sorêt band	
624	572	535·5	501	406	mμ

This suggested uroporphyrin and an authentic specimen of uroporphyrin I, when examined in chloroform in the same spectrophotometer, gave maxima at:

I	II	III	IV	Sorêt band	
624	570·5	535	501	406	mμ

These peaks show very good agreement, as may be seen in Fig. 1. A mixture of uroporphyrin I octamethyl ester and the *Duvaucelia* porphyrin ester were made in equal proportions as far as possible and examined in the spectrophotometer. (If two dissimilar substances are examined spectrophotometrically the peaks of the absorption curve are usually very rounded and the curve presents an undulating instead of a sharp appearance.) The maxima in this case were:

I	II	III	IV	Sorêt band	
624	570·5	535	501	405	mμ

The peaks of the curve (Fig. 1) are very sharp and the curve suggests the presence of one pure substance only.

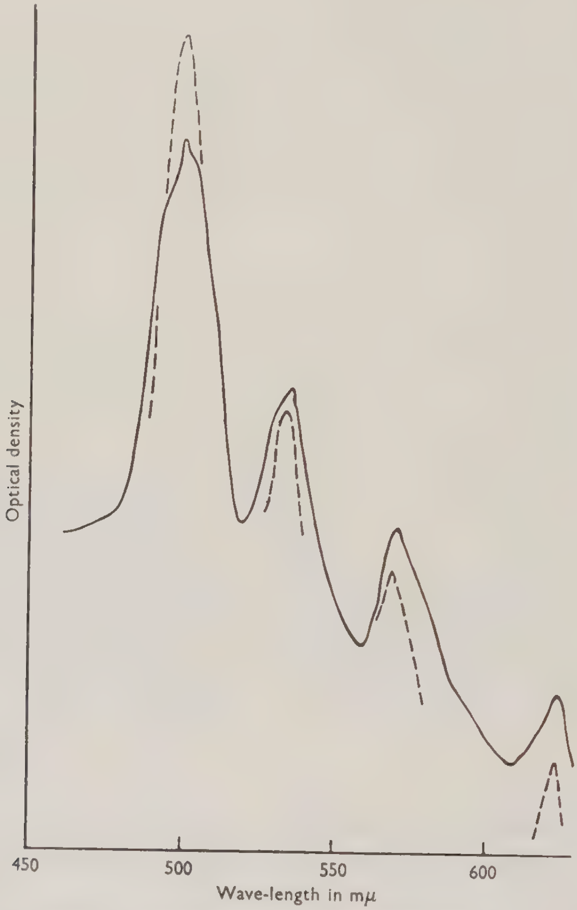

Fig. 1. Absorption spectra of *Duvaucelia* porphyrin in chloroform. ——, absorption spectrum of *Duvaucelia* porphyrin alone; - - - - -, absorption spectrum of a mixture of *Duvaucelia* porphyrin and authentic uroporphyrin I.

A little of the porphyrin ester solution from *Duvaucelia* was evaporated to dryness and hydrolysed by dissolving it in 7N-HCl and standing at room temperature for 36 h. At the end of this time the excess acid was removed by placing the dish in a vacuum desiccator over solid potassium hydroxide until dry. The residue was dissolved in a little chloroform, spotted on a long paper (Kennedy, 1953*b*) and developed at 23° C with 2:6-lutidine/water (5:3) in an atmosphere of ammonia. The resulting chromatogram showed a single spot at R_F 0·15, indicating a porphyrin with eight carboxyl groups. This test was repeated using pure uroporphyrin I as a marker. With mixed spots only one large spot appeared at R_F 0·15 with a small spot above it at R_F 0·09 (the 'second spot' of uroporphyrin). In chromatograms with authentic uroporphyrin I as marker, parallel spots formed at R_F 0·15 and one small spot on the marker side at R_F 0·09.

These results together with the absorption spectrum confirm that the *Duvaucelia* porphyrin is uroporphyrin. The uroporphyrin was characterized as isomer I by the two-dimensional paper chromatogram technique of Falk & Benson (1953), and (at Sheffield) by its decarboxylation to coproporphyrin I identified by the Chu, Green & Chu (1951) technique.

The Porphyrin of Aplysia punctata

The upper integument was carefully removed from fifteen *Aplysia punctata*, all collected from Looe by Dr B. C. Abbott, to whom we are indebted for his kindness. The material was washed in several changes of fresh water to remove as much as possible of the 'aplysiopurpurin' pigment. The integuments were then dried roughly on filter-paper and extracts made in exactly the same way as for *Duvaucelia*. The final chloroform extract was evaporated to dryness and redissolved in dry chloroform and chromatographed on magnesia grade III (Nicholas, 1951). The material quickly resolved itself into a series of bands as follows, from the top downwards:

(1) Greyish.
(2) Brown-purple.
(3) Green.
(4) Blue.
(5) Purple (red fluorescent).
(6) Yellow (blue fluorescent).

It proved impossible to separate bands 5 and 6 from one another, and they were collected together. The other bands, although interesting, were discarded for the present, to be investigated in the future. The red fluorescent chloroform extract containing bands 5 and 6 was evaporated to dryness, to give a fatty residue. This was redissolved in dry chloroform and the absorption curve determined in the spectrophotometer. The following maxima were obtained:

I	II	III	IV	Sorêt band
624	570	534	501	406 mμ

This strongly suggested uroporphyrin, an authentic specimen of which gave the maxima described under the *Duvaucelia* pigment.

Some of the *Aplysia* porphyrin ester was hydrolysed by standing in 7N-HCl for 48 h and the acid removed by standing over solid KOH *in vacuo* overnight. The residue was dissolved in 2:6-lutidine and examined in the long-paper chromatograph in a 2:6-lutidine/water system in an atmosphere of ammonia at 23° C. Three papers were run, including the *Aplysia* porphyrin alone, and with authentic uroporphyrin I as marker in mixed and adjacent spots. On all papers two spots were obtained at R_F 0·09 and 0·15, indicating that the porphyrin of *Aplysia* was a uroporphyrin.

The Chu *et al.* (1951) technique, followed by that of Falk & Benson (1953) established the uroporphyrin as isomer I, confirmed (at Sheffield) by its conversion to coproporphyrin I.

The Porphyrins of Luidia ciliaris

The upper integument of the starfish *L. ciliaris* was extracted in the usual way with a methanol-sulphuric acid (19:1) mixture and the porphyrin brought into chloroform. After evaporation to dryness on the water-bath this extract was redissolved in dry chloroform and chromatographed on magnesia grade III (Nicholas, 1951). The column was monitored by ultra-violet light. A blue fluorescent band of fatty material passed rapidly down the column and was discarded. Two purplish bands, both red fluorescent, then appeared and passed very slowly down the column about 2 cm apart. These were eventually eluted with chloroform-methanol (100:3) (band 1) and chloroform-methanol (100:5) (band 2) respectively.

Band 1. This was identified as protoporphyrin by spectrophotometry in pyridine (including comparison with authentic protoporphyrin) and by conversion to mesoporphyrin.

Band 2. This band in chloroform solution was evaporated to dryness in portions. One of these was dissolved in pyridine and examined in the spectrophotometer. The following maxima were obtained:

I	II	III	IV	Sorêt
648	590	562	519	425 mμ

The curve obtained was clearly of the rhodo-type (that is, the optical density of the peaks of absorption decreased in the order III, IV, II and I). This fact, together with the positions of the maxima of absorption, strongly suggested chlorocruoroporphyrin. The oxime was prepared (at Sheffield) and a spectrophotometric examination in pyridine showed a characteristic shift of the absorption maxima towards the blue:

I	II	III	IV
638	580	548	507 mμ

This is good evidence for the presence of a formyl (—CHO) group. Authentic chlorocruoroporphyrin, prepared from *Myxicola–Sabella* blood, gave an oxime with maxima at:

I	II	III	IV
639	579	543	507 mμ

To determine the presence of vinyl groups, the porphyrin was treated with diazo-acetic ester followed by hydroxylamine. This produced a shift to the blue, but the rhodo-type spectrum was retained. The maxima in pyridine were:

I	II	III	IV
645	586	557	516 mμ

This is consistent with the presence of one vinyl group, and is confirmatory evidence for the porphyrin being chlorocruoroporphyrin.

As an additional check on the identity of the main porphyrin of *Luidia*, the following experiments were carried out.

Fresh *Luidia* tissue was extracted by the ether : acetic acid procedure, and after the usual purification, the porphyrins were esterified by standing dissolved in methanol : HCl for 48 h at 0° C. The esters, after purification, were dissolved in dry chloroform and separated by chromatography on a column of alumina packed in a mixture of equal parts of chloroform and ether (Lemberg & Parker, 1952). The band of protoporphyrin dimethyl ester passed rapidly down the column well in advance of the main band of chlorocruoroporphyrin ester, and was discarded.

The chlorocruoroporphyrin band was collected, filtered, and the solvent removed *in vacuo* without heat. The residue was dissolved in glacial acetic acid saturated with sodium chloride and the chlorocruorohaematin prepared, the iron being introduced by the method of Paul (1950).

The chlorocruorohaematin was converted into the haemochromogen by the method of Warburg, Negelein & Haas (1930), viz.: the haematin was dissolved in aqueous pyridine (pyridine 1 ml. : water 3 ml.) and one-tenth of the volume of hydrazine hydrochloride solution added (hydrazine hydrochloride 0·7 g in 5 ml. water + 5 ml. of 2N-NaOH). The haemochromogen gave the following bands in the Hartridge Reversion Spectroscope:

I	II	Intensity
584·4	545·9 mμ	I>II

This result agrees well with that obtained for chlorocruorohaemochromogen by Lemberg & Falk (1951):

I	II	Intensity
583·1	545·1 mμ	not given

The haemochromogen from the 'haem *a*' obtained from ox-hearts by Rawlinson & Hale (1949) gave only *one* band in the visible spectrum at 587 mμ (Lemberg & Falk, 1951; Falk & Rimington, 1952; Rimington, Hale, Rawlinson, Lemberg & Falk, 1949).

These experiments distinguish clearly between the main porphyrin obtained from *Luidia* and the porphyrin *a* from haem *a* of heart muscle. In addition, the spectrum of the *Luidia* porphyrin—chlorocruoroporphyrin—is of the rhodo-type—intensities III, IV, II, I—whereas that of the porphyrin *a* is of the oxorhodo-type—intensities III, II, IV, I. The porphyrin in *Luidia* occurs free, and not as a haem.

The Porphyrins of Astropecten irregularis

The integuments of fifty specimens of *Astropecten irregularis* were carefully stripped off and extracted with methanol/sulphuric acid overnight in the usual way. The pigments were taken into chloroform and the extract washed and evaporated by the conventional technique. The evaporated chloroform extract was redissolved in dry chloroform and chromatographed on a column of magnesia grade III (Nicholas, 1951), developing with chloroform with increasing concentrations of methanol, as for *Luidia*. The same type of chromatogram was obtained, and the two red fluorescent bands were shown to be protoporphyrin III type 9 and chlorocruoroporphyrin in the same way and by the same tests as were applied to the porphyrins of *L. ciliaris*. *Astropecten*, however, appeared to have much less porphyrin (and particularly protoporphyrin) in the integument than *Luidia*, although no quantitative investigation was carried out at this stage.

Discussion

This survey of a number of marine invertebrates from eight phyla shows that porphyrins are present in eight species for certain (with one doubtful species *Tethya aurantium*), and that these eight species belong to only three phyla—Annelida, Mollusca and Echinodermata. Porphyrins were not found in any of the coelenterates, crustaceans or tunicates examined, although MacMunn (1887) found haematoporphyrin in the madreporarian corals *Flabellum variabile* and *Fungia symmetrica*.

The finding of uroporphyrin in the upper soft integument of *Duvaucelia plebeia* (better known as *Tritonia*) and *Aplysia punctata* is of considerable interest in view of the presence of this pigment in some mollusc shells (Nicholas & Comfort, 1949). MacMunn (1887) found 'haematoporphyrin' in the black slug *Arion empiricorum* (a synonym introduced by Férussac, Férussac & Deshayes, 1819–51, to cover the numerous colour and pattern varieties of the slug more generally known as *A. ater*). Thus the only molluscs which have been shown definitely to contain a porphyrin in their soft parts are either species without shells (*Duvaucelia* and *Arion*) or species with uncalcified shells (*Aplysia*), whereas uroporphyrin, with traces of coproporphyrin, occurs in several of the shells of shell-bearing species. This suggests that in molluscs the porphyrin is normally laid down in the shell (an integumentary product), or failing that in the uncalcified integument. The former would be in keeping with the deposition of uroporphyrin I in the bones and teeth in congenital porphyria, and in the bones of the Pennsylvanian fox-squirrel *Sciurus niger* (Turner, 1937). Turner connected the formation of the uroporphyrin I with the megaloblasts of the bone marrow, and postulated that in *Sciurus* there exists a unique persistence into the adult span of the normal method of foetal haemoglobin synthesis.

Protoporphyrin III type 9 occurs in the shells of the eggs of the hen and of a plover (Fischer & Kögl, 1924). Borst & Königsdorfer (1929), Fikentscher (1932), Fraenkel (1924) and Hammer (1930) found that small amounts of porphyrin occur in the bones of the human foetus, as well as in those of newborn babies and other newborn mammals, mainly in the centres of ossification. It is also interesting to recall the experiments of these workers with growing animals, in which injected uroporphyrin I and haematoporphyrin III type 9 were quickly deposited, the latter being required in large amounts before deposition takes place. Bingel (1937) showed that uroporphyrin III was also deposited in bones and teeth. Coproporphyrin I, mesoporphyrin III type 9 and protoporphyrin III type 9 appear to have a slight tendency to deposit in this way. The bones of the foetus do not become impregnated with porphyrins injected into the mother, according to Borst & Königsdorfer (1929) and others, but Kench, Langley & Wilkinson (1953) have shown that porphyrins are transmitted through the placenta but are rapidly excreted. This is surprising in view of the known affinity of one porphyrin at least—haematoporphyrin—for actively growing tissues (Figge, Weiland & Manganiello, 1948; Kennedy, 1952). The deposition of porphyrins in the asteroids is perhaps somewhat similar, although it is difficult to demonstrate whether the porphyrins are present in the spicules or not, owing to the difficulty of separating the spicules from the integument without destroying any porphyrin they might contain. The presence of free chlorocruoroporphyrin in *Luidia ciliaris* and also in *Astropecten irregularis* is of great interest, since this porphyrin has hitherto only been found to occur naturally in the form of its haemoglobin, chlorocruorin, in the blood of sabellid worms (Fox, 1926, 1949). Lemberg & Legge (1949) have suggested that 'since this pigment is found only in a small group of worms which live in the same type of environment as do others containing erythrocruorin with protohaem IX as prosthetic group, the peculiarity does not appear to be of adaptive importance, and may be an evolutionary relic'.

In the three species of echinoderms shown to contain porphyrins, *Asterias rubens* has only protoporphyrin (Kennedy & Vevers, 1953*b*) which has two vinyl groups, while *Luidia ciliaris* and *Astropecten irregularis* have chlorocruoroporphyrin, which has one vinyl and one formyl group. *Luidia ciliaris* and *Astropecten irregularis* also have protoporphyrin in the integument. Warburg (1932) considered that the presence of a carbonyl (=CO) group in a side chain (which later becomes a vinyl group) is a primitive characteristic. The occurrence of chlorocruoroporphyrin in *Luidia* and *Astropecten*, both phanerozonian asteroids, may therefore be regarded as an additional argument for classifying the Phanerozonia as less specialized than the Forcipulata such as *Asterias rubens* (Grassé, 1948, p. 237). Apart from this, however, it would appear that the distribution of integumentary porphyrins in starfishes is not only random in relation to their taxonomic position but also in relation to their ecology and mode of life. Thus the presence of protoporphyrin in *A. rubens* and its absence in *Marthasterias glacialis* is surprising, for not only are these two starfishes classified in the same family (Asteriidae), but their larvae are almost identical in form and scarcely distinguishable (Mortensen, 1927), and the adults feed in the same way on the same type of food. In areas where their geographical ranges overlap, as they do off Plymouth, these two species may, in fact, be said to occupy the same ecological niche.

In the polychaetes the porphyrin is probably present in the viscera, but this is less certain as it is often difficult to separate integument from viscera in sufficient quantity for analysis. In *Chaetopterus*, in which the digestive tract was separated from the rest of the animal for extraction, the porphyrins certainly came from the viscera.

SUMMARY

A survey of forty-eight species of British marine invertebrates has shown that porphyrins which have not previously been described in these animals are present in three representatives of the Annelida, two of the Mollusca and two of the Echinodermata. The occurrence of protoporphyrin in *Asterias rubens* was confirmed in a previous paper (Kennedy & Vevers, 1953*b*). A red-fluorescent extract was obtained from the sponge *Tethya aurantium*, but it contained too little material for further investigation. No trace of a porphyrin was found in the coelenterates, crustaceans and tunicates examined, although Moseley found 'polyperythrin' (which MacMunn later identified with the 'haematoporphyrin' of *Asterias* and *Arion*) in *Flabellum* and *Fungia*.

Chlorocruoroporphyrin was found free in the starfishes *Luidia* and *Astropecten*. Uroporphyrin was extracted from the soft dorsal integument of *Duvaucelia* (= *Tritonia*) *plebeia*, and from the integument of *Aplysia punctata*, observations of interest in view of the widespread occurrence of uroporphyrin in mollusc shells.

Coproporphyrin III was characterized from extracts of the viscera of *Myxicola infundibulum*, *Nereis diversicolor* and *Chaetopterus variopedatus*. Evidence of the occurrence of a penta-carboxylic porphyrin was obtained from paper chromatography of the extract from the gut of *Chaetopterus*.

These findings are discussed in comparison with the distribution of porphyrins in birds and mammals.

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J. Mar. biol. Ass. U.K. (1954) 33, 677–679
Printed in Great Britain

PROTOSTYELA HETEROBRANCHIA N.GEN., N.SP., A STYELID ASCIDIAN FROM THE SCOTTISH WEST COAST

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(Text-fig. 1)

The ascidian described in this paper was collected on 22 April 1952 from the shore of the island of Luing, Argyll. It was taken, along with colonies of *Didemnum maculosum* (Milne Edwards), from the level of low water of spring tides, on a part of the shore which, although very sheltered from waves, is subject to fast tidal currents. Only a single zooid was found, but it differs so clearly from all known ascidians that it is being made the type of a new genus and new species. The following description applies to the zooid after it had been stored in 10% formalin for nearly two years.

DESCRIPTION

The body is about 2·0 mm long, ovoid, and very slightly depressed perhaps owing to contraction (Fig. 1 A). It is pale brown and has a smooth surface with no incrusting material. There is a narrow ventral area of attachment and a small basal extension of the test which spreads over the substratum and which in life was probably linked to other zooids. The test is thin and moderately tough, semi-transparent and marked with small brown spots. A circular area round each siphon is without pigment. Both siphons are round, without lobes, and are so short that they scarcely project from the surface of the body. The body-wall is thin and almost transparent and possesses very slender muscles which are visible only under high magnification. They form an open irregular mesh except on the siphons, which have radial muscles. There are about twelve simple oral tentacles of alternating sizes. The branchial sac (Fig. 1 C) is very characteristic. It possesses no folds but on each side are three narrow longitudinal bars, the dorsal one on the right side being very close to the dorsal lamina. No transverse bars are present. The stigmata, of which there are about sixteen rows, are transverse, instead of longitudinal as in almost all other ascidians. They are long rather wide rectangular openings and number about three in each transverse row. The dorsal lamina is an undivided membrane. Details of the small dorsal tubercle were not seen. A short curved oesophagus leads to the barrel-shaped stomach which has eight to ten complete longitudinal folds (Fig. 1 B). The stomach and intestine lie to the left of the branchial sac. The intestine forms a narrow simple loop bending back close to the stomach. No pyloric caecum was seen but one may possibly be present. The short flattened rectum turns forward to end in the smooth-edged elliptical anus. On the right side of the body there are three gonads (Fig. 1 D), which are attached to the body-wall and form a row parallel to the endostyle but a short distance from it. The posterior gonad (Fig. 1 E) is hermaphrodite and consists of an undivided dorsal testis and a ventral ovary.

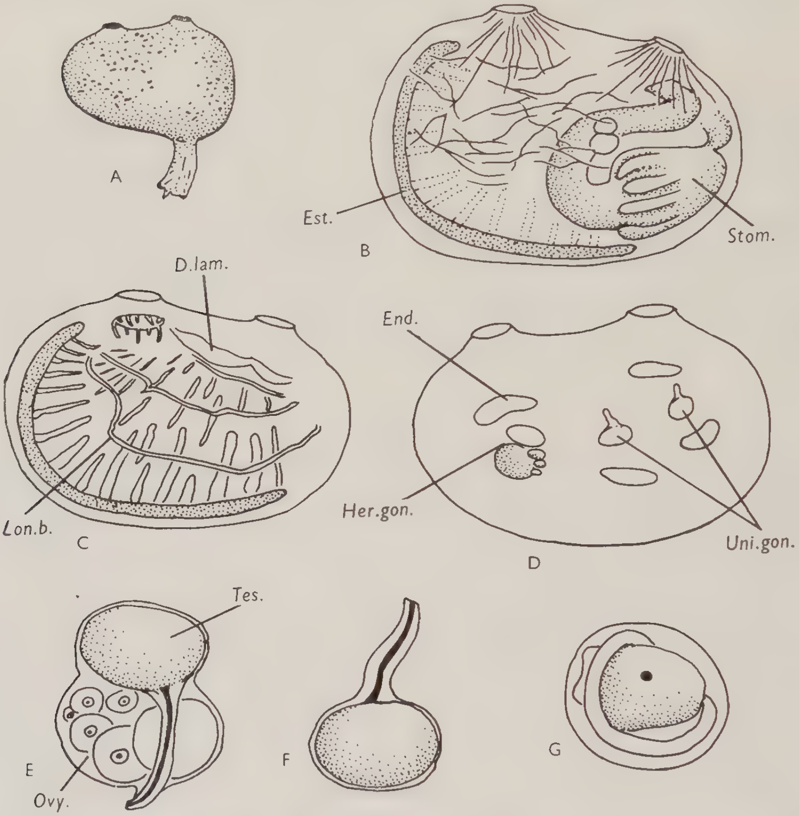

Fig. 1. *Protostyela heterobranchia.* A, zooid from right; B, zooid from left, with test removed; C, zooid from left, dissected to show right half of branchial sac; D, zooid from right to show gonads; E, hermaphrodite gonad; F, unisexual (male) gonad; G, larva. *D.lam.*, dorsal lamina; *End.*, endocarp; *Est.*, endostyle; *Her.gon.*, hermaphrodite gonad; *Lon.b.*, longitudinal bar; *Ovy.*, ovary; *Stom.*, stomach; *Tes.*, testis; *Uni.gon.*, unisexual (male) gonad.

The sperm duct passes down across the mesial surface of the ovary and opens immediately ventral to it. No oviduct was distinguished. In the two anterior gonads (Fig. 1 F) only a simple testis was seen and these gonads are almost certainly unisexual. The sperm duct of each of these two male gonads is directed dorsally. On the left side of the body there is also a hermaphrodite gonad like that on the right, but other gonads were not seen. A few ovoid endocarps are present on each side of the body.

Some developing eggs and one apparently nearly fully developed larva (Fig. 1 G) were found in the atrial cavity, and the species is therefore viviparous. The developing eggs have a diameter of about 0·23 mm measured over the chorion. The trunk of the larva is about 0·22 mm long and has at its anterior end a triangular projecting structure which represents the three adhesive papillae. A single black sense organ is present. The tail of the larva was still coiled round the trunk and therefore could not be measured.

The new species, although represented by a single zooid, clearly belongs to the group of compound styelid ascidians sometimes recognized as the subfamily Polyzoinae (Hartmeyer, 1903). In many species of this group the colony consists of zooids closely united within a common test. In others, however, the zooids are widely separated and united only by a basal creeping stolon or sheet of test material, and this is apparently the condition in *Protostyela heterobranchia*.

In possessing transverse stigmata the genus differs from all known compound styelids except *Berrillia* (Brewin, 1952), but *Berrillia* is distinguished by having four longitudinal bars on each side of the branchial sac, and by the presence of gonads on the left side only.

Protostyela n.gen. may be defined as follows: a genus of the family Styelidae forming colonies of loosely united zooids; branchial sac without folds; stigmata transverse; hermaphrodite and unisexual gonads present in the same zooid.

The geographical distribution of *P. heterobranchia* is still unknown, but its apparent absence from the intensively collected areas of the Channel suggests that it may have a more northerly range. This possibility is strengthened by the presence of larvae in April long before high summer temperatures are reached.

SUMMARY

Protostyela heterobranchia n.gen. n.sp. is a compound styelid ascidian from Scottish waters. The branchial sac has three longitudinal bars on each side and no folds, and the stigmata are transverse. Both hermaphrodite and unisexual (male) gonads are present.

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J. Mar. biol. Ass. U.K. (1954) 33, 681–687
Printed in Great Britain

THE BREEDING AND DEVELOPMENT OF THE ASCIDIAN *PELONAIA CORRUGATA* FORBES AND GOODSIR

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(Text-figs. 1–3)

Pelonaia is a genus of the family Styelidae (subphylum Tunicata; class Ascidiacea) which is very distinct, anatomically, from all other ascidians. It appears, however, to be closer to *Styela* than to other genera of the family. The only known species, *Pelonaia corrugata* Forbes & Goodsir, has a mainly circumpolar arctic distribution and extends for a short distance southward into boreal waters. In British waters it occurs chiefly round the Scottish coast, and is not definitely known south of the Firth of Clyde and Cullercoats. Within its geographical range *Pelonaia* occurs only locally, being restricted to bottoms of sand or mud. The loose nature of the substratum has had a great influence on the form of the body, which is long, narrow and worm-like. *Pelonaia* is not attached to a solid object, but instead lives upright in the sand or mud, with the lower part of the body embedded and the upper part, which bears the siphons, projecting freely into the water. The shape and habitat are unlike those of most sand-dwelling ascidians which are usually globular and are often anchored in the surface layer of the sand by filaments of the test.

The development of *P. corrugata* was unknown and was interesting for two reasons: first, the species has a rather isolated position in the family Styelidae, and secondly, the specialized habitat of the adult might be expected to affect the method of development.

Material and Methods

Specimens were dredged from sandy mud at depths of 40–47 m, off Fairlie in the Firth of Clyde. Fertilized eggs were obtained in two ways: (1) by allowing animals kept in aquarium tanks to liberate eggs and sperm spontaneously, the eggs being removed to Petri dishes after fertilization; and (2) by taking eggs and sperm from the ducts of animals within a few hours of collection, and adding sperm from one individual to eggs from another. Developing eggs were kept in covered Petri dishes or watch-glasses containing freshly collected unfiltered sea water. The water, which was changed once daily, maintained a temperature of 8·0–10·0° C. A few embryos were fixed in Boveri's picro-acetic mixture, embedded in paraffin wax (m.p. 49° C.), and sectioned at 8μ.

OBSERVATIONS

The Breeding Season

Specimens were collected at about monthly intervals between August 1950 and February 1954. In most samples the population could be separated by inspection into two groups representing different age classes. There therefore appeared to be a limited breeding season. Fig. 1 shows the distribution by body volume of the specimens taken on 11 January 1954. Animals up to about 1·0 ml. were about 1 year old and those between 1·2 and 2·4 ml. about 2 years old. Only the animals in the older group had fully developed and functional gonads at this time of year. The breeding season was found by examining the specimens in the monthly samples, and noting when the gonads started to empty and when they had completely emptied. In 1952 spawning had not yet started on 17 January, but was completed before 25 March. In 1953 spawning had just started on 12 January, and was finished by 26 January. In 1954 spawning started between 15 January and 22 January and ended about 15 February. The breeding season in this area is therefore confined to a period of 2–4 weeks in January or February. During breeding in 1954 the bottom temperature over the spawning animals was about 7·5° C., and animals kept in an aquarium tank spawned at a temperature of about 7·0° C. Thus *P. corrugata* breeds, in the Firth of Clyde, when the sea is almost at its coldest, a fact correlated with the mainly arctic distribution of the species. The southern limit of its world distribution coincides roughly with the February isotherm of 7·2° C. (Fig. 2); higher temperatures further south probably limit distribution by preventing breeding.

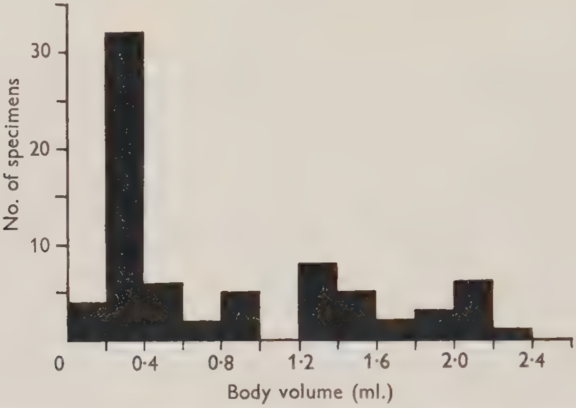

Fig. 1. Distribution by body volume of the specimens of *P. corrugata* collected on 11 Jan 1954

The ripe ovum (Fig. 3A) is spherical, bright orange in colour, and from 270 to 280μ in diameter. It is surrounded by a thin chorion forming a spherical transparent shell 300 to 320μ in diameter. The outer follicle cells, which are rounded and have no vacuoles, cover the chorion. Inner follicle cells are inconspicuous. Much yolk is present and the egg has a high specific gravity. Consequently the eggs of *Pelonaia* drop to the bottom when shed into the

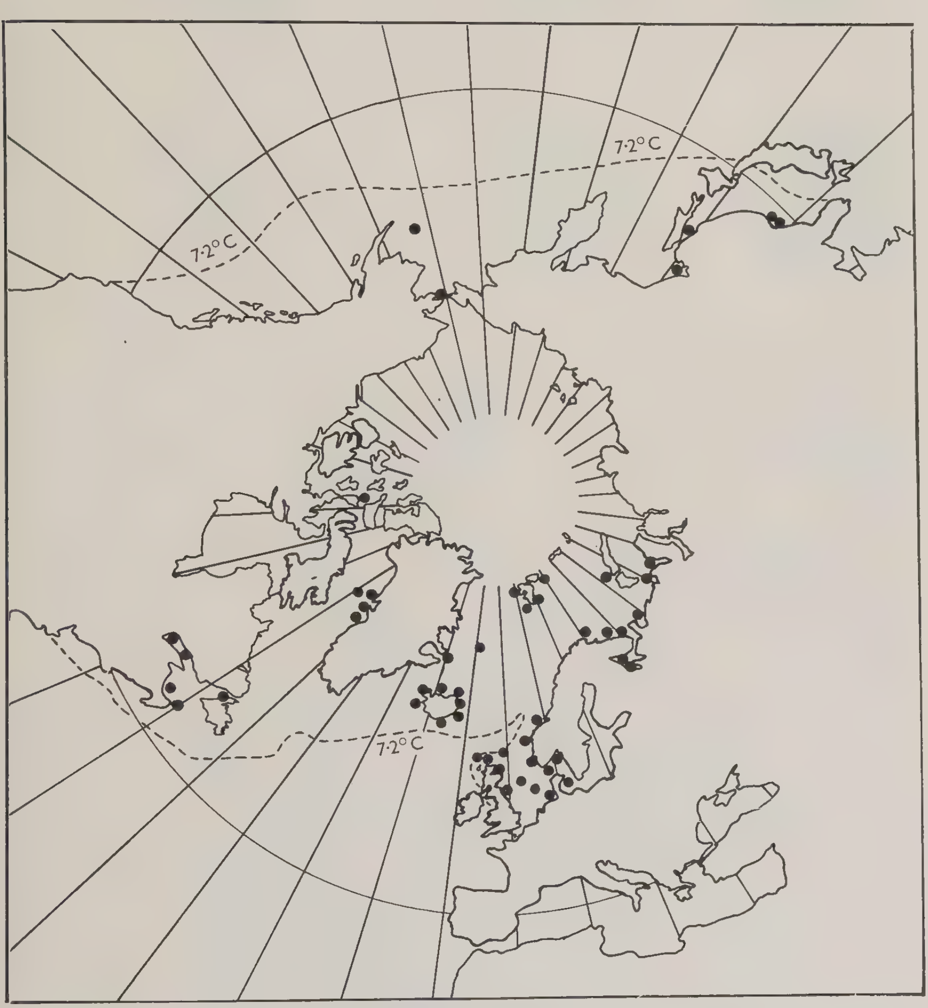

Fig. 2. Known geographical distribution of *P. corrugata*, showing also the February isotherm of 7·2° C.

water, instead of remaining suspended as do the eggs of many ascidians. This is important in connexion with the mode of development, as will be seen later.

The sperm has the shape common in ascidians. The narrow rod-like head about 12 μ long has, near one end, an asymmetrical protoplasmic bulge, and arising from this end, a tail about 62 μ long.

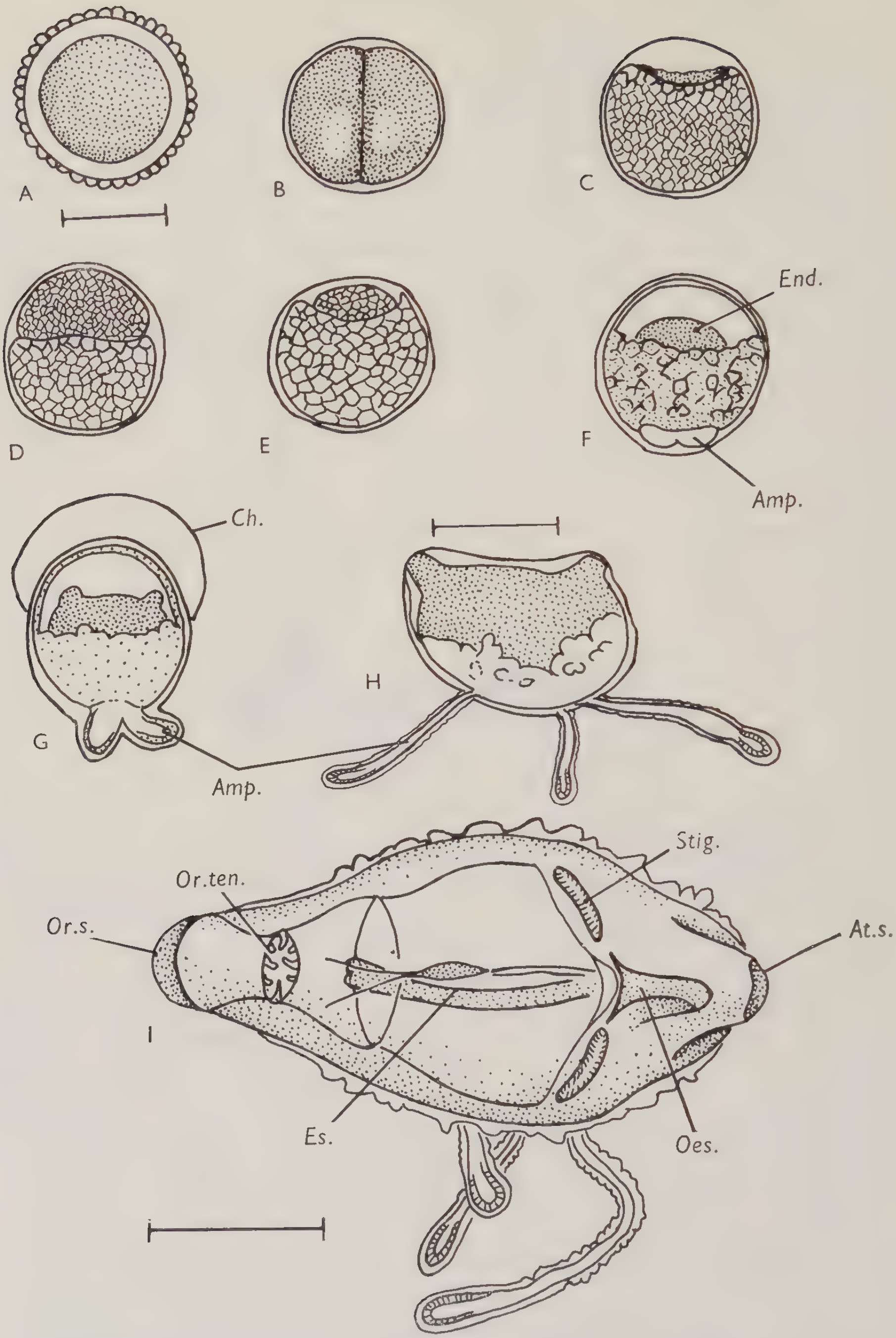

Fig. 3. Development of *P. corrugata*. A, unfertilized egg; B, two-cell stage, showing area of pale protoplasm in each cell; C, gastrula; D, two-hemisphere stage, the lower hemisphere consisting of large pale cells and the upper one of small dark cells; E, later two-hemisphere stage; F, embryo showing rudiments of two ampullae; G, rupture of chorion; H, embryo after attachment; I, young ascidian. D-H are lateral views and I a dorsal view. In B–G the outer follicle cells have been omitted. The scales represent 200 μ. *Amp.*, ampullae; *At.s.*, atrial siphon; *Ch.*, chorion; *End.*, endoderm; *Es.*, endostyle; *Oes.*, oesophagus; *Or.s.*, oral siphon; *Or.ten.*, oral tentacles; *Stig.*, stigma.

Embryonic Development

Artificial fertilization was attempted each month for over 2 years, but succeeded only in January 1953 and January and February 1954. At other times the sperm was almost completely inactive. Only a few, and usually less than 12, of the eggs taken from any animal became fertilized. There were therefore not enough embryos to allow fixation and sectioning of all the most important stages, and the following outline of development was obtained mainly from the study of live embryos.

Cleavage of the fertilized egg is symmetrical and is like that of other ascidians up to the time of gastrulation. The gastrula (Fig. 3C) is formed within about 12 hr. of fertilization at a temperature of 8·0–10·0° C. There is little localization of coloured protoplasm into presumptive areas, such as occurs in some styelid ascidians and occasionally amongst other families. In the 2- and 4-cell stages, however, each cell shows a pale area in the otherwise uniformly coloured protoplasm (Fig. 3B), and in the 16-cell stage there is a group of four dark cells. Later stages show no colour pattern. Following gastrulation the embryo rounds off and remains spherical for about a day. During the next 2 days the spherical stage is transformed into a two-hemisphere stage (Fig. 3D). In this, one half of the embryo consists of large pale orange cells and the other half of small dark orange cells. The hemisphere of large cells now slowly grows and begins to envelop the other hemisphere, which meanwhile shrinks (Fig. 3E). At the end of the process, usually 4–6 days after gastrulation is complete, the embryo has an internal ovoid mass of small dark cells surrounded by a sphere, part of which is still recognizable as the large pale cells of earlier stages. The internal dark mass is endoderm and the surrounding sphere ectoderm. The ectoderm at one point now differentiates and by rapid growth forms a pair of blister-like swellings, which are the rudiments of two ampullae (Fig. 3F). The whole development so far has taken place within the chorion, which however is now ruptured by the elongation of the ampullae and by the swelling due to the secretion of test over the whole ectoderm (Fig. 3G). The ampullae grow rapidly, and by secreting sticky test substance, fix the embryo to the substratum. The swollen ends of the ampullae have tall epidermal cells and are regions especially active in the secretion of test. In the culture vessels the embryos became attached to the glass by means of the ampullae, but in nature they would presumably be fixed to sand grains by the same mechanism. At this point in development the central endodermal mass shows the rudiments of the two siphons projecting outwards towards the enclosing ectoderm. The remaining central mass is the rudiment of the pharynx and gut. The orientation of the future ascidian can now be established: the ampullae arise from the ventral surface and the siphons mark the dorsal surface. In most specimens a third ampulla appears at this stage (Fig. 3H), but in some only two are present throughout development. The siphons are now completed by fusion of the endodermal rudiments with the ectoderm, which also enters into their formation. The endostyle and dorsal lamina appear in the pharynx, and the oesophagus and other parts of the gut become visible. Branchial stigmata are not formed until some time later; in one individual the first pair appeared on the 20th day after fertilization, but in others not until the 40th day. The young ascidian (Fig. 3 I) now has its essential organization and is soon able to feed.

Discussion

A few attempts have been made to relate the geographical distribution of ascidians with sea temperatures (Hartmeyer, 1923–24; Thompson, 1930–34; D. & A. Carlisle, 1954) but very little is known of the exact way in which temperature limits the range. We do not know in any particular species, for instance, whether high summer temperature or low winter temperature is a limiting factor, and whether the boundaries of distribution are fixed by inability of the adult to survive, or to breed successfully, beyond the temperature limits. In *Pelonaia* it appears that the southern boundary is fixed by the failure of breeding in higher winter temperatures farther south. It would be interesting to know the breeding period in northern waters, and we would expect it to be shifted progressively towards the summer in the more northerly parts of the range. Although the limiting factor at the northern boundary of the range is unknown, it may well be a winter temperature too low for survival of the adult. If this is so *Pelonaia* illustrates the type 4 geographical zonation, as defined by Hutchins (1947); in this type of distribution both the poleward and the equatorward limits are set by the winter temperature.

The distribution of the species is further limited by the need of a substratum loose enough to allow the adult to be partially embedded, but not subject to shifting with the consequent danger of burial. Such a habitat, however, is local, and the species may require for survival not the wide dispersal of larvae but rather their retention in the area already inhabited by the adults. The pelagic larval stage which is normal in other styelids and indeed in most ascidians, has been eliminated in *Pelonaia*. Moreover, the egg is heavy, and probably sinks to the bottom instead of drifting in water currents. The young stages therefore develop and are established close to the breeding parent stock. In the elimination of a pelagic larval-stage *Pelonaia* presents a striking parallel with the sand-dwelling species of the Molgulidae. Berrill (1931) has shown that in this family the sand-flat habitat is common and is usually accompanied by anural development. He regards the suppression of the larval stage as correlated with the habitat although he does not consider 'that such a habitat is directly responsible for its origin, but that conceivably through a lessening of the rigour of natural selection, those species in which it does arise are allowed to survive, whereas among attached shore forms they are not'. But the occurrence of anural development in the genus *Pelonaia*, which although unrelated occupies a similar habitat, suggests that this type of development is of positive survival value.

In this investigation I have not been able to make a detailed study of the embryology, but intend to do so in the future, as several very interesting problems are involved. First, there is the fate of the tissues which in urodele forms produce the tail. Secondly, the larval sense organs (ocellus and otolith) fail to develop, but we do not know whether the larval nervous system is totally suppressed, and if so, how the adult system arises. Thirdly, the stages intermediate between the gastrula and the hatching embryo are obscure, particularly the two-hemisphere stage which requires an explanation in terms of cell-lineage. Fourthly, what is the relation between those changes leading to the formation of the young ascidian stage and the changes which occur in the metamorphosing larva of urodele species?

I wish to thank the skipper and crew of M.F.V. *Calanus* for their help in collecting material, and my wife for her care of the cultures.

SUMMARY

Pelonaia corrugata in the Firth of Clyde breeds for only 2–4 weeks in January or February. The species is oviparous. Early cleavage is similar to that of other ascidians, but after gastrulation the embryo passes through a two-hemisphere stage. One of these hemispheres is endodermal. From the other hemisphere, which slowly envelops the endoderm, two ampullae grow and fix the embryo to the substratum. The young ascidian develops directly from this attached embryo, and the development is therefore anural. Elimination of the pelagic larval stage is regarded as an adaptation to the sand-dwelling habit of the adult, as it prevents dispersal to unsuitable habitats.

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J. Mar. biol. Ass. U.K. (1954) 33, 689–697
Printed in Great Britain

THYROIDECTOMY OF THE DOGFISH, *SCYLLIUM CANICULA* (L.), AND THE EFFECT OF DOGFISH THYROID UPON THE OXYGEN CONSUMPTION OF RATS

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(Text-fig. 1)

The function of the thyroid gland in fish is still obscure in spite of the numerous attempts to elucidate it by means of the administration of thyroid extracts, thyroxin, thyrotropin and antithyroid drugs, as well as by the carrying out of direct histological and cytological observations upon the gland at various seasons of the year and phases of the life cycle (Goldsmith, 1949; Hopper, 1952). The influence of this gland upon the metabolic rate of higher vertebrates is well established, but, despite the recent demonstration that the teleost thyroid contains a principle which raises the oxygen consumption of mammals (Smith & Brown, 1952), most attempts to influence the rate of oxygen consumption in fish themselves by the use of thyroid stimulants or depressants have been negative (Root & Etkin, 1937; Etkin, Root & Mofshin, 1940; Hasler & Meyer, 1942; Smith & Everett, 1943), the only positive result being that of Smith & Matthews (1947), who injected extracts of the Bermuda parrot fish into the Bermuda white grunt.

Studies of the growth and maturation of thyroid-treated fish have also been contradictory (Hoar, 1951; Hopper, 1952; Smith, Sladek and Kellner, 1953), and while chemical thyroidectomy appears to inhibit growth (Goldsmith, Nigrelli, Gordon, Charipper & Gordon, 1944; Nigrelli, Goldsmith & Charipper, 1946) and gametogenesis (Barrington & Matty, 1952), the possibility remains, as the latter authors point out, that these results may be due to some action of the drug which is not mediated by the thyroid gland.

Despite the fact that surgical removal of the glands has proved an invaluable procedure in endocrine research, thyroidectomy has not been carried out in teleosts, which have been mainly used in these studies, probably because the gland is usually diffuse and intimately related to the ventral aorta. Thus a lack of information regarding the influence of the absence of their own gland upon the oxygen consumption of fish remains a serious gap in our knowledge, and it has seemed essential to attempt to fill this by devising a procedure for surgical thyroidectomy in elasmobranchs in which group the distinct and encapsulate nature of the gland presents a more promising situation, as was realized by Vivien (1941). The present paper describes such a procedure, and presents the results of observations upon the oxygen consumption of fish after thyroidectomy. For comparison with these results the influence of elasmobranch thyroid on the oxygen consumption of male white rats has also been studied.

MATERIALS AND METHODS

Operative Technique

Dogfish of lengths ranging from 60 to 70 cm and of both sexes were used in all the operations, the animals being first anaesthetized by placing them in a 1·5% solution of urethane in sea water for 15 min. After washing in tap water they were placed ventral side uppermost in an operating cradle. The head was strapped down over the oro-nasal grooves on to the operating board and the lower jaw held back by retractors, thus exposing the floor of the mouth. A half-inch transverse incision was made through the mid-line in the fold of loose skin lying immediately behind the teeth of the lower jaw. After loosening the connective fascia between the coracomandibular and corocohyoids these muscles were held apart by small stainless steel retractors in order to expose the thyroid gland. This narrows anteriorly into a long 'thyroglossal cord' which terminates in the region of the symphysis of Meckel's cartilages. The anterior end of the 'thyroglossal cord' was first severed from its connective tissue attachment and the intact gland removed in an anterior-posterior direction, the region of the anterior attachment and also the region of the posterior attachment of the gland to the muscle lying dorsal to the coracohyoids being cauterized locally with an ophthalmic cautery in order to destroy any stray follicles that might have been left by the operation. The duration of the operation varied from 20 to 30 min; there was no appreciable haemorrhage, and the animals recovered consciousness almost immediately after being replaced in sea water. After some operations the sinus left by removal of the gland was packed with absorbent sterilized gelatin sponge. Nylon thread was used to stitch the skin incision, and the normal aseptic operating conditions were employed.

After a few days all the operated animals began to feed upon strips of fresh squid muscle. An autopsy was performed on every thyroidectomized fish after the completion of the respiration experiments; in no case was thyroid tissue identified macroscopically. The mortality rate was satisfactorily low, no deaths having occurred in the experiment, to be described later in detail, in which the oxygen consumption of operated animals was determined. Operated dogfish have, in fact, been kept alive for a period of at least 72 days after the complete removal of the gland.

A vertical approach through the throat was attempted in some preliminary experiments, but this method was abandoned when it was found to result in nearly a 100% mortality within a week or so.

Determination of Oxygen Consumption of Dogfish

As a result of the experience gained from some preliminary work carried out in the summer of 1952, using the Winkler technique for the determination of dissolved oxygen, it was decided to use the polarographic technique in the definitive experiments, in order to make possible the handling of a large number of fish. The respiration apparatus was of the continuous flow type similar in principle to that described by Hall (1929). Each dogfish was placed separately into a respiration chamber consisting of a bitumen-painted compressed asbestos pipe, 15 cm in diameter and 76 cm long. The ends of each chamber were made of Perspex plates which could be removed for inserting the fish, and through which it was possible to observe the movements of the animals in order to investigate the possibility of activity influencing the oxygen consumption during the metabolism experiments. No appreciable activity was in fact observed. A constant flow of water maintained at 400 ml./min was allowed to pass through the chamber containing the animal for 8 h. Four chambers were used simultaneously. The temperature of the tank of water surrounding the respiration chambers, which varied during the experiment from 16·2 to 17·6° C, was recorded daily.

As mentioned above, oxygen content of the water was measured by the polarographic method, the apparatus used being of the manually operated type with a dropping mercury electrode (Milton & Waters, 1949; Giguère & Lauzier, 1945). Samples of the inflow and outflow water, taken between 5 and 7 p.m. each day, were run directly into 20 ml. polarographic cells, and their oxygen contents measured at 16·5° C. (The sampling error of duplicate samples of water was always less than 1·0%.)

Determination of Oxygen Consumption of Rats

Twenty adult male albino rats were used in this study, their weights ranging from 185 to 260 g. Four rats were used as untreated controls, their oxygen consumption being measured every day for 6 days. Four more control rats were injected intra-peritoneally with 2·0 ml. of 0·9% sodium chloride in distilled water, whilst in a further group of eight each animal was given a single injection of 100 mg of mammalian thyroid powder. The remaining four were each given a single injection of 95 mg of dried dogfish thyroid. This dogfish thyroid was prepared by placing the fresh gland in acetone at 4° C and then washing several times in acetone to remove the fats. Finally the glands were washed in alcohol, dried in a desiccator and ground up into a fine powder.

The four rats which received saline injections were at a later date each given a single injection of a suspension of 100 mg dried trout muscle.

The oxygen consumption measurements were made using the method of Grad (1952), except that 9,000 ml. desiccators were found to be more convenient than the original stoppered jars as respiration chambers.

All determinations were made between 9.30 and 11.30 a.m. at a temperature of 28 ± 0·5° C. During the experiment between the periods in which they were in the respiration chambers, the animals were kept supplied with abundant food and water.

RESULTS

Oxygen Consumption of Thyroidectomized Dogfish

The oxygen consumption of thyroidectomized dogfish was measured individually on three male and three female animals from the 1st to the 42nd day after the operation, at 3-day intervals. Six animals, three male and three female, given sham operations consisting of the complete operative procedure except for the removal of the gland, were used as controls, their oxygen consumption being also measured at 3-day intervals. Both of these groups of fish were measured before the operations in order to determine the normal oxygen consumption of untreated fish. The results expressed as the mean oxygen consumption of the six fish in each of the two groups are given in Table I. They show that there is no significant decrease in the oxygen consumption of either group for a period of 42 days after the operation, nor is there any significant difference between the oxygen consumption of the two groups when these are compared every 3rd day.

TABLE I. THE EFFECT OF THYROIDECTOMY ON OXYGEN CONSUMPTION OF *SCYLLIUM CANICULA*

Six fish in each group (* Five fish only in group). In the first column a minus (−) sign indicates days before operation, a plus (+) sign days after operation.

	Thyroidectomy		Sham operation		
Days	Mean O_2 consumption (ml./g/h)	S.E.	Mean O_2 consumption (ml./g/h)	S.E.	'*t*'
− 5	47·8*	3·02	48·0*	1·00	0·056
− 2	49·3	3·96	47·5	2·97	0·644
+ 1	48·3	3·33	42·5	1·52	1·446
+ 4	48·3	3·52	42·8	3·85	0·962
+ 7	45·8	2·70	43·7	1·92	0·578
+10	47·0	3·07	44·2	3·00	0·596
+13	44·2	3·44	47·2	3·76	0·537
+16	51·0	3·98	55·2	2·98	0·784
+19	51·6	3·54	52·0	3·50	0·073
+22	49·8	4·57	50·0	3·16	0·033
+26	49·3	3·86	45·8	2·38	0·704
+31	46·5	3·55	—	—	—
+36	47·7	2·84	—	—	—
+39	47·2	2·86	44·3	1·87	0·775
+42	44·2	3·73	—	—	—

The Effect of Dogfish Thyroid upon the Oxygen Consumption of Adult Rats

The results given in Fig. 1 are expressed as average percentage change in oxygen consumption. The values of oxygen consumption for the various test series before injection gave a total mean of 1·37 ml./g/h (S.E. ± 0·039) which is seen to be in close agreement with the mean oxygen consumption of 1·33 ml./g/h (S.E. ± 0·039) determined on a completely separate series of twenty-four animals. The mammalian thyroid preparation caused a maximal increase in oxygen consumption of 26·1 %, 5 days after the injection; whilst the dogfish thyroid preparation gave a maximal response of 24·8 %, 4 days after the injection. Since the degree of response is about the same for approximately the same level of dosage it is probable that both preparations are of similar strength.

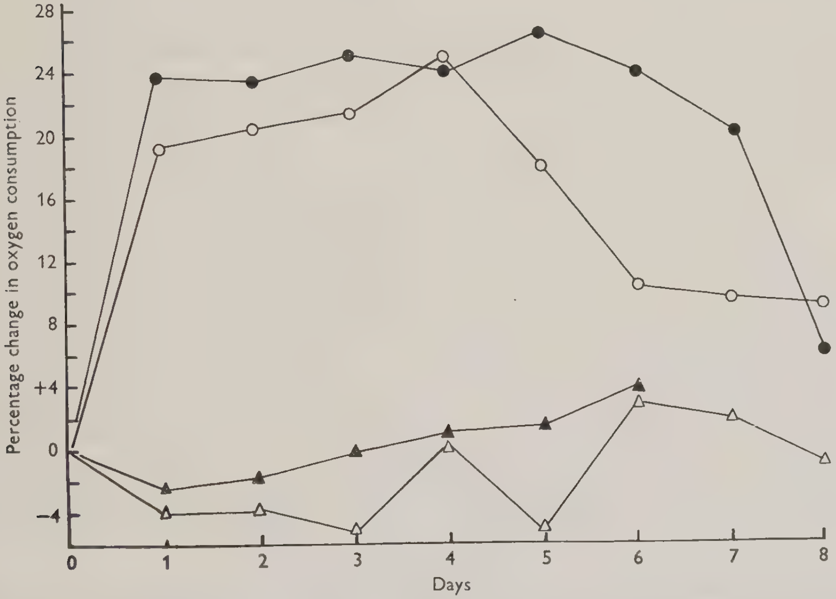

Fig. 1. Percentage change in oxygen consumption of rats injected intraperitoneally with thyroid preparations. △, normal controls; ▲, injected with 2·0 ml. 0·9 % NaCl; ●, injected with 100 mg mammalian thyroid powder; ○, injected with 95 mg dogfish thyroid.

The saline-injected rats showed no increase in oxygen consumption either before or after injection with dried trout muscle.

Discussion

Gorbman, Lissitzky, Michel & Roche (1952), from radio-active iodine chromatographic studies on *Scyllium* thyroids, have shown that di-iodotyrosine and thyroxine are formed in the thyroid gland of these animals whilst the present experiments have demonstrated that dried dogfish thyroid gland when injected intraperitoneally into rats is capable of increasing their oxygen consumption. These investigations indicate clearly that there is present in the dogfish thyroid a thyroxine-containing hormone similar to that found in mammals. Furthermore, as thyroidectomy of the dogfish has been shown above to cause no change in the oxygen consumption of the whole animal for a period of up to 42 days it may be concluded that although the thyroid hormone is present it has no influence over the immediate oxygen consumption of the dogfish. In the teleosts the evidence for a thyroxine-containing thyroid hormone is meagre, for although Smith & Brown (1952) have shown that a teleost thyroid preparation is capable of increasing oxygen consumption in rats, and Matthews & Smith (1947*a*) have demonstrated the presence of radio-active iodine uptake by the thyroid no chemical analysis of the gland has appeared in the literature. Also surgical removal of the gland appears never to have been attempted.

The technique of surgical thyroidectomy stands in marked contrast to that of 'chemical thyroidectomy' using thiourea or similar drugs as a thyroid-inhibitor, for the immobilization of the gland after the latter treatment is known not to take place until some time after the commencement of treatment, whilst total removal of the gland at once prevents any further supply of the hormone to the animal. This consideration of the use of goitrogens certainly applies, for example, to the work of Matthews & Smith (1947*b*) who injected *Fundulus* with 0·5–1·0 mg of thiourea per gram of fish daily for a period of up to 6 days and were unable to detect any significant change in oxygen consumption. The possible toxic effects of goitrogens during prolonged immersion or injection experiments of fish are also obviated by thyroid removal; that such toxic effects might occur in fish is evident from the discussion of goitrogens and enzymes by Salter (1950).

Although surgical thyroidectomy appears to have advantages over the chemical thyroidectomy approach for the investigation of thyroid function both suffer from the fact that the investigations so far reported, including the present work, may not have had the experiments prolonged for sufficient period for delayed effects on oxygen consumption to become evident. Thyroidectomy or goitrogen treatment of mammals results in a significant decline in oxygen consumption within some 5 days, but does not generally produce a maximal depression of basal metabolic rate until 2 or 3 weeks after the commencement of treatment (Siebert & Smith, 1930; Barrett & Gassner, 1951), and as the rate of oxidative metabolism in poikilotherms is known to be lower than that of homoiotherms the rate of response in the former might be expected to be substantially slower. Moreover, the fact that the temperature of poikilotherms is generally lower than that of homoiotherms might also effect the speed of response, for it has been demonstrated in fish that the thyroid gland shows cellular hypertrophy during treatment with thiourea more rapidly at high than at low temperatures (Fortune, 1953). Another possibility to be taken into account in evaluating the present results is the fact that the animal may be able to synthesize extra-thyroidally a similar hormone to that produced by the thyroid. Preliminary experiments have shown the presence of protein-bound radio-active iodine in the plasma of dogfish which have been thyroidectomized for at least 6 weeks (Matty, unpublished). It might be supposed that if the extra-thyroidal hormone maintained the normal oxygen consumption level in thyroidectomized dogfish then treatment of such animals with anti-thyroid drugs for some period might prevent any production of the hormone and so cause a fall in the oxygen consumption, assuming, of course, that these drugs also inhibit the extra-thyroidal synthesis. Until the results of such further work are reported the possibility of extra-thyroidal hormone supply is not eliminated but, as in the higher vertebrates where the conditions of complete thyroxine deficiency after thyroidectomy is difficult to obtain (Leblond & Eartly, 1952), such investigations initially depend on surgical thyroidectomy.

Having established the presence of a thyroxine-containing hormone in dogfish which does not have any immediate effect on the oxygen consumption of the whole animal the search for identifying the functions of the thyroid must be directed elsewhere. According to Waring, Landgrebe & Bruce (1942), thyroids removed from the dogfish hypophysectomized a month previously afford no evidence for pituitary control of thyroid activity. There is some indication of a correlation between reproduction and thyroid activity in teleosts (Barrington & Matty, 1952; Robertson & Chaney, 1953; Scott, 1953), and there is evidence also that the activity of the gland is increased in elasmobranchs during the breeding season (Guariglia, 1937; Zenza, 1937). Some observations during the present work have been in conformity with these latter reports, but unfortunately there is as yet no direct experimental evidence for a relationship between the thyroid gland and the reproductive activity of elasmobranchs.

Another possible function of the thyroid is that it is concerned with the early stages of growth and maturation of fish. Experiments on teleosts have shown that on immersion in goitrogens growth is retarded (Hopper, 1952; Smith *et al.*, 1953), but as Smith *et al.* have pointed out the effect may be a non-physiological toxic one, and, as already mentioned, efforts to accelerate growth of teleosts by thyroxine, thyroid or thyrotropin treatment have given most equivocal results. The situation in elasmobranchs is even more obscure, for no studies on the possible relationship between the thyroid gland and growth in these animals seem to have been reported. It is, however, hoped that the use of surgical thyroidectomy, as described in the present work, will provide a useful basis for investigating some of these outstanding problems of thyroid relationships in the elasmobranchs.

My thanks are due to Prof. E. J. W. Barrington for his advice and encouragement, and to Mr A. Aitken, for the benefit of continued discussion throughout this work. The elasmobranch experiments were carried out at the Marine

Biological Laboratory, Plymouth, with financial support from the University of Nottingham, and I am indebted to the Director and his staff for their unfailing co-operation.

SUMMARY

Surgical thyroidectomy has been successfully performed on the dogfish, *Scyllium canicula*, and has been shown to have no signficant effect on the animal's oxygen consumption over a period of 6 weeks.

The injection of extracts of dogfish thyroid into adult male white rats results in an increase in the animal's oxygen consumption of the same order as that produced by mammalian thyroid preparations.

The significance of these results is discussed in the light of recent investigations of thyroid function in fish.

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J. Mar. biol. Ass. U.K. (1954) 33, 699–708
Printed in Great Britain

ON THE DIVERSE COLOURS OF *NEREIS DIVERSICOLOR*

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(Text-figs. 1 and 2)

The variable colour of the polychaete *Nereis diversicolor* has been known since O. F. Müller described the species as 'Die bunte Nereide' in 1771. Most of the worms belonging to this species appear to be orange or brown in colour, though close examination reveals that some green pigment is invariably present. However, others may be found which are predominantly green in appearance, and a few which are completely green and appear to lack brown or orange pigments in the epidermis.

Several previous authors have given attention to this. Mendthal (1889) considered the green colour of some worms to be due to a diet of green algae. McIntosh (1910) rejected this idea, and suggested that the green colour was due to 'pale greenish ova'. However, it may be pointed out that males are also green, and in our experience the oocytes of this species are colourless or have only a pale straw colour. Various other writers have commented on the variability of the colour of this animal without contributing to the elucidation of the problem, Thomas (1930) alone has attempted an analysis of the different pigments. He concluded that the green pigment was probably a porphyrin, possibly a modified chlorophyll. Herpin (1923, 1925) and Dehorne (1925), as well as Thomas (1930) and Dales (1950), have drawn attention to the rather different appearance of the two sexes when mature. Hempelmann's paper (1939) on the chromatophores of *Platynereis dumerilii* has little bearing on the present problem.

Most of the chemical work described here has been done on worms from Plymouth in 1953 and 1954, but observations on variation of colour have been made mainly at Chalkwell, Essex, over the last five or six years, and some chemical analyses have also been made on worms from this locality.

IDENTIFICATION OF THE PIGMENTS

In one extract, twenty-five whole worms were placed, without grinding, in absolute methanol 19 parts and concentrated sulphuric acid 1 part, overnight in the ice chest. The following day the deep-green extract was filtered through glass wool into a separating funnel, and diluted with an equal volume of water.

The mixture was then extracted with one-third of the volume of chloroform. The chloroform hypophase was dark brown, and the aqueous epiphase was bright green. The hypophase was transferred to another funnel and washed with 2 % NaCl and then with water, forming 'chloroform extract A'. The aqueous epiphase was again extracted with chloroform, and this time the hypophase was bluish green and the epiphase pale green. The hypophase was removed and washed as before, filtered through chloroform-soaked paper, and evaporated to dryness *in vacuo*. A greenish residue remained which was used for subsequent tests; this was 'chloroform extract B'.

Chloroform Extract A

Extract A was evaporated to dryness *in vacuo* and redissolved in dry chloroform. The solution was chromatographed on ungraded Brockmann's alumina packed in chloroform, and then exhibited the following bands:

Band 1. A dark green band at the top of the column; non-fluorescent.

Band 2. A narrow yellow band; also non-fluorescent.

Band 3. A reddish brown band which travelled rapidly down the column and passed out. This was strongly red-fluorescent and gave the following spectrum (Hartridge Reversion spectroscope):

	I	II	III	IV
	660–680	600–620	590–565	520– mμ
Centres	670	610	579·5	(520)

Bands 1 and 2 could not be eluted by any of the usual solvents or combinations of solvents, and were finally eluted by ether-acetic acid 5:1 mixture, giving a green solution with no fluorescence and very indeterminate spectrum. The pigment had the extraordinarily high HCl-number of 36.

Band 3 was further chromatographed on MgO grade III (Nicholas, 1951), packed in chloroform, and displayed the following bands:

Band A2. A dark green band at the top; non-fluorescent.

Band B2. A brown band, also non-fluorescent, separated from the upper band by a gap.

Band C2. An apparently colourless wide band, red-fluorescent, passing down and out of the column very readily.

*Band A*2 (Fig. 1)

This band when cut off and eluted with methanol displayed the clear spectrum of biliverdin:

I	II
639	390 mμ

and when acidified with HCl there was a shift of the bands to:

I	II
678	375 mμ

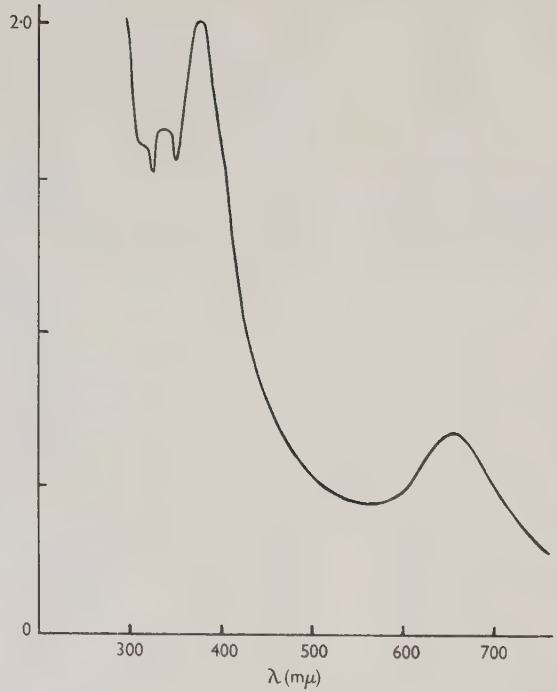

Fig. 1. Absorption curve of an acetic acid extract of the body-wall of entirely green worms. Vertical scale in arbitrary (Unicam spectrophotometer) units.

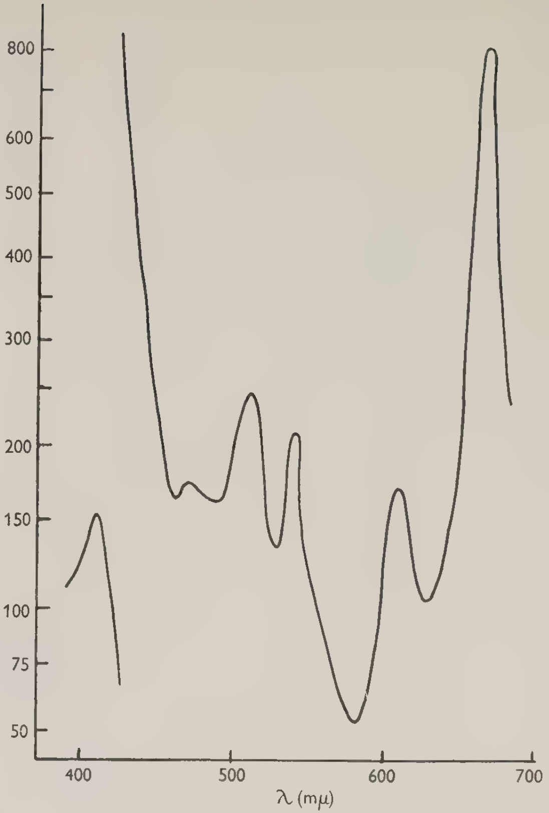

Fig. 2. Absorption curve of a chloroform extract of whole worms. Vertical scale in $E_{1\text{cm.}} \times 10^3$.

Band B2

This was eluted with MeOH: $CHCl_3$ 1:3 mixture, but gave no clear spectrum.

Band C2 (Fig. 2)

This band, when concentrated in chloroform solution, gave a clear spectrum in the spectrophotometer of the chlorin type, with maxima at

I	II	III	IV	V	Sorêt
666	609	540	507	(468)	414 mμ

which is almost identical with the spectrum of methyl phaeophorbide-*a*:

I	II	III	IV	Sorêt
666	610	535	506	412 mμ

Chloroform extract B

The residue from this extract was found to give the spectrum of biliverdin with methanol and methanol/HCl:

	I	II
Methanol	639·4	391·2 mμ
Methanol/HCl	678·6	375·4 mμ

The acid-number was 1.

The solution of the pigment in methanol/HCl was allowed to stand for 36 h and then extracted with chloroform in the usual way, followed by crystallization from chloroform-methanol. Fine blue-green needles were obtained, melting at 210° C, and giving a mixed melting-point with pure biliverdin dimethyl ester of 207° C.

The pigment was confirmed as biliverdin by the formation of zinc bilipurpurin; by the van den Bergh reaction; by giving a negative result with Ehrlich's diazo reaction; and by its destruction with concentrated sulphuric acid (which distinguishes it from mesobiliverdin).

Paper chromatography

The extracts used for paper chromatography were obtained from larger quantities of worms and these were pulped before extraction.

Paper chromatography of a chloroform extract on long papers (Kennedy, 1953) at 23° C with 2:6 lutidine-water in an atmosphere of ammonia revealed the presence of a porphyrin with four carboxyl groups and a green pigment with one carboxyl group only, according to the technique of Nicholas & Rimington (1949). The tetra-carboxylic porphyrin was shown to be coproporphyrin III by applying the technique of Chu, Green & Chu (1951), and employing an authentic specimen of pure coproporphyrin III tetramethyl ester. The green monocarboxylic porphyrin pigment gave the same spectrum as that obtained from the band C2 already mentioned. Since the extract from which C2 was obtained was made by using methanol and concentrated sulphuric acid, the pigment of band C2 would be an ester. From its solubility in boiling absolute ethanol, and its acid-number of 16, the pigment appeared to be methyl phaeophorbide-*a* only. Hydrolysis of the ester and formation of derivatives—phylloerythrin, phaeoporphyrin-a_5 and phytorhodin-*g*—confirmed that this was so. Extraction of the ether solution of the pigment with 0·2% sodium bicarbonate was without result, proving the absence of phaeophorbide-*b* (Willstätter & Stoll, 1913). That the pigment is present in the worm as the free phaeophorbide-*a* is shown by the formation of the spot of R_F 0·94 on the long-paper chromatogram of worm extracts made in chloroform only without any esterification.

As compared with the quantity of biliverdin, phaeophorbide-*a* is present in small amounts and may indeed be restricted to the gut. Worms thus owe their green appearance to biliverdin, while the yellow, brown and orange pigments also present in the epidermis in most worms are carotenoids.

DISTRIBUTION OF THE PIGMENTS IN THE BODY

In most worms biliverdin occurs in the form of minute granules 1–2 μ in diameter scattered through the epithelial cells immediately under the cuticle. The pigment is deposited mainly along the borders of the blood capillaries, and is therefore most dense on the dorsal side of the body and in the parapodia; but it also occurs at the bases of the parapodia and between the segments. The appearance of the worms suggests that there is less biliverdin in the most anterior part of the body on the dorsal side, and while this seems to be so, the darker appearance is due mainly to the much larger amounts of carotenoids in this region. This darker appearance of the anterior region of the body may have some significance in relation to survival of these worms which protrude from the burrows while browsing on the surface mud. Biliverdin does not usually occur in any quantity on the ventral side of the body except in the parapodia, but depositions are found in the pygidium, and under the cuticle lining the proboscis. However, the intestine and the coelomic cells are also usually faintly green and contain small quantities of similar granules of biliverdin, and in females which have wholly or partially spawned, the gut and the coelomic cells may present a vivid green appearance. All worms possess at least some biliverdin though its presence may be masked by yellow, orange or brown carotenoid pigments.

Some individuals may appear entirely green, biliverdin alone being represented in the epidermis. These worms are always either ripe males with free sperm in the coelom, ripe females, or females which have spawned. This does not mean that females with an orange appearance are not sexually mature or do not spawn. Such green individuals, whether male or female, owe their striking appearance not only to a disappearance of the orange and brown carotenoids from the skin, but also to an increase in the amount of biliverdin in the body.

On the other hand, slight variations in the 'greenness' of worms which are not breeding seem to be due to variations in the quantity of carotenoids, worms appearing greener when they have relatively small amounts, and more orange when they have relatively greater quantities of carotenoids. On the other hand, apart from the breeding worms, there seems to be little variation in the amount of biliverdin deposited in the epidermis throughout most of the year.

SEASONAL CHANGES IN COLOUR

In the Chalkwell population during the early spring of 1949, nearly all the worms assumed a predominantly green appearance by the end of February when the main spawning occurred (Dales, 1950). It was naturally concluded that this colour change was in some way related with the maturation of the gametes. This is now known to be only partly true, since subsequent experience has shown that the same population in other years, and populations which have been seen elsewhere at the time of spawning, may have only a relatively small number of green individuals. Other observers have also reported the rather variable occurrence of green specimens, which as a general rule constitute only a small proportion of any population. During the summer such green individuals are definitely rare. In the early spring of 1951, 1952, and 1954 there were few green worms at Chalkwell, but in the winter and spring of 1952–53 there was again a much larger proportion of worms with a green appearance, but by no means as many as in 1948–49.

DISCUSSION

Breeding takes place in mid-February in the *Nereis diversicolor* population at Chalkwell, but only a very small proportion of the females spawn successfully owing to the relative scarcity of the males and the fact that spawning takes place only in the presence of the male. All males with mature sperm appear a bright green owing to the complete extraction of the orange and brown pigments and also to an increase in the relative amount of biliverdin, as already mentioned. This was shown to be so by the much greater amount of pigment which could be extracted per unit weight of such worms as compared with non-breeding animals. This increase in the total quantity of biliverdin is almost certainly associated with a reduction in the quantity of haemoglobin (in the sense of Keilin & Hartree, 1951). As the males reach maturity their tissues undergo phagocytosis, the body-wall muscles are eroded away, making the worm extremely fragile. As this process continues the amount of haemoglobin decreases; there is often barely enough blood to fill the dorsal vessel in the fully mature male. This suggests that the biliverdin is indeed derived from the worm's own haemoglobin.

On the other hand, females when apparently mature at the time of spawning are more variable in appearance; some are predominantly green, but all at this time have quantities of orange or brown pigments as well as the biliverdin in the body-wall. Later, however, those females which have spawned lose their orange and brown pigments and become as green as mature males. Males soon die when they have emitted their sperm. Females that have spawned often live for several weeks after spawning. Females rarely spawn so completely that one or two oocytes are not left in the coelom enabling their sex to be determined with certainty, and all such females undergo a phagocytosis of the tissues during or after spawning, accompanied by a loss of carotenoids and a greatly increased destruction of the blood haemoglobin with a corresponding increase in the quantity of biliverdin in the body. In the female the process of phagocytosis seems to have got out of step with the spawning, and this rather anomalous situation may be related in some way to the loss of the epitokous phase in this species. In unspawned females the oocytes are eventually broken down by the coelomic cells, being converted into a milky mass which might be mistaken for sperm. Soon afterwards quantities of orange pigments appear in the body-wall. These worms live throughout the following summer and may double their length, but it seems unlikely that they have a second opportunity to spawn (see Dales, 1951). There is no doubt that the green female individuals are those which have spawned, even if an appreciable number of oocytes remains in the coelom. Spawning takes place in the burrows and the worms are remarkably sedentary, at least at this time of year. For the first 8 weeks at least the larvae develop in the parent burrow and are just visible to the naked eye when about 6 weeks old. Larvae are invariably found in burrows occupied by a spawned or partially spawned green female, or in empty burrows, but never in burrows occupied by orange worms in which the oocytes are being absorbed. The explanation of these colour differences may be simply a consequence of sexually mature worms ceasing to feed. Worms that spawn subsequently draw on their carotenoids and even on their muscular tissues and become green and fragile. Worms that do not spawn, on the other hand, have a large reserve supply of food in the form of their own oocytes, and when these are absorbed the worms become even more orange in appearance than before. If the carotenoids form part of food reserves, the occurrence of greater numbers of green individuals in winters with early cold periods (1948–49, 1952–53) could be due to the earlier depletion of these stores. On the other hand, the carotenoids may be utilized directly in the production of the gametes, but if this is so it is surprising that though the carotenoids disappear in the male at maturity, they may not disappear in the female until after spawning. Incidentally, the greater number of green individuals in the early spring of 1949 was certainly not due to a larger proportion of males or spawning females in that year. Counts to determine the sex ratio in early February 1949 showed that less than 10 % of the total population were males, and this proportion has not varied materially in subsequent years. The proportion of spawned females has also remained at approximately the same value.

Although it is difficult to observe or measure changes in the quantity of epidermal biliverdin in adults which already have variable amounts of green, orange and brown pigments, it was thought that some indication of whether the biliverdin was derived from ingested blood or not could be solved by rearing worms from the larval stage on different substances. Young worms were collected in large numbers from the burrows in early May when the average age was estimated at about 8 weeks. At this time there are about ten to twelve chaetigerous segments and they are completely colourless. These young worms were reared on (1) dried and powdered nettle leaves alone, (2) dried slaughterhouse blood alone, and (3) a dried concentrated aquarium fish-food ('Brosiam') containing negligible amounts of chlorophyll or haemoglobin. The young *Nereis* seemed to be sufficiently omnivorous to thrive well on each of these substances for at least long enough for the present purpose. The water in each culture dish was replaced daily with filtered sea water to ensure that the worms were in fact feeding on the diet provided. The haemoglobin appeared in the blood when the young worms were about 9 weeks old, and the other pigments appeared soon after. The orange and brown pigments first made their appearance as granules in stellate chromatophores concentrated in the anterior region and arranged as a double row down the body on the dorsal side. The biliverdin appeared at about the same time, and from the first was more uniformly scattered through the epidermal cells on the dorsal side. All the young worms were similarly pigmented after several weeks' growth on these different foods; those fed entirely on nettle leaves, and those on dried blood, had much the same quantity of epidermal biliverdin as those fed on the dried fish-food.

Thus there is little doubt that the biliverdin is formed by the breakdown of the haemoglobin of the blood. This takes place along the margins of the vessels, mainly between the capillaries on the dorsal side of the body around the proboscis and in the pygidium. Bloch-Raphaël (1939) concluded, in her review of the seat of haemoglobin synthesis and breakdown in polychaetes, that in nereids these processes probably take place in the body-wall and around the proboscis, and that the bile pigments are excreted into the gut. This view agrees with the present observations on *N. diversicolor*. The granules of biliverdin may gradually be removed from the epidermis by the coelomic cells and conveyed to the gut, the cells travelling down the septa and oblique muscles. Coelomic cells loaded with biliverdin, often in granules 4–5 μ across, can be seen in these positions in spawned females. When the rate of haemoglobin breakdown is increased as in ripe males and spawned females, the rate of elimination is not increased in proportion, so that the pigment accumulates in the body.

SUMMARY

The variable colour of *Nereis diversicolor* is due to variations in the proportion of green, orange and brown pigments. The orange and brown pigments are mainly carotenoids; the green colour is due to biliverdin.

Phaeophorbide-*a* and coproporphyrin III also occur, but both these pigments may be restricted to the gut wall; biliverdin occurs both in the wall of the gut, and in the epidermis and coelomic cells.

The biliverdin is formed by the breakdown of the haemoglobin of the blood.

Haemoglobin-breakdown takes place in the epidermis on the dorsal side of the body, in the epithelial tissue surrounding the proboscis and in the pygidium. Granules of biliverdin are probably removed by the coelomic cells and conveyed to the gut into which they are excreted.

In ripe males, and in females during and after spawning, phagocytosis of the tissues is accompanied by an increased haemoglobin-breakdown with a corresponding accumulation of biliverdin in the body. The green appearance is due not only to an increased amount of biliverdin, but also to a complete extraction of carotenoids from the body-wall.

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J. Mar. biol. Ass. U.K. (1954) 33, 709–719
Printed in Great Britain

INNERVATION OF AN AMPHIPOD HEART

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(Text-figs. 1 and 2)

For investigation of the innervation of the heart in the Amphipoda, *Marinogammarus* marinus* has been chosen as it is among the largest species available at Plymouth. The smaller size of other species added greatly to the difficulties in making the preparations. Some observations were made with *Gammarus locusta*, but because of certain features of its anatomy referred to below it was found to be less suitable for this kind of study.

For staining of the nerves methylene blue was used. The immersion of the tissues into a weak solution of this dye (10–15 drops of 0·5% solution in distilled water to 100 ml. of sea water) gave more constant results than the injection of a stronger solution into the body of the animal. However, the staining of certain elements, such as the nerve cells in the ganglionic trunk, could be obtained with the latter method only. The individual reactions of the animals to the staining varies greatly: thus, when several specimens are injected with the same solution at the same time the nerves of some stain more or less well while for others the results are poor or often nil.

To expose the heart the ventral body wall of the animal should be cut along the middle line or, to avoid damage to the ventral neural cord, along the side of the latter. After the parts of the sectioned body wall have been pulled out and pinned to the paraffin plate, the digestive organs are removed, leaving *in situ* the two dorsal diverticula which are so linked with the posterior aorta that it is practically impossible to separate them from it. The gonads should also be removed. The digestive organs are connected with the pericardial diaphragm by strands of connective tissue, but there is no particular difficulty in severing these connexions and in obtaining preparations with the heart tube and the pericardial diaphragm in their normal positions. In *G. locusta*, however, this diaphragm seems to be a little weaker and is more easily damaged; the heart tube then becomes flaccid or even displaced, and subsequent manipulations are much more difficult.

Such preparations with the heart and the pericardium left *in situ* show many of the details described below, but for better observation of heart nerves the heart tube should be opened along its middle line. This operation can be done with a very fine scalpel and preferably after some staining has made the outlines of the tissue elements more distinguishable. After the heart has been opened its ganglionic trunk is more easily accessible for staining and observation, but even then, being situated on the outside of the dorsal heart wall, it is seen by transparency only. For its direct observation the heart has to be isolated and mounted with the dorsal side up. This is a difficult operation, and it is more expedient to perform it at one of the later stages, after the preparations have been stained and fixed and are already either in water or alcohol or xylol.

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* Separated from *Gammarus* by Sexton & Spooner (1940).

The fixation of the staining has not been quite satisfactory with the tissue of *Marinogammarus*. As is already known, the elements stained with methylene blue, to be well fixed with ammonium molybdate solution, must attain a certain intensity of colour. In gammarids some of the nerves do not reach this point, and although they show up distinctly before being fixed they turn pale or even become indistinguishable in the completed preparations. Neither was the double fixation, i.e. in ammonium picrate followed by ammonium molybdate, of much help. With such material the best practice is, after having examined the permanent preparations, to check repeatedly the dubious points on fresh preparations during the staining process before their fixation.

The Anatomy of the Heart

The heart of *Marinogammarus* has the shape of a tube extending from the posterior edge of the first thoracic segment (which enters into the composition of the cephalothorax) to the middle of the 7th thoracic segment. Its wall is made up of a layer of cross-striated muscle fibres turning in left-handed spirals surrounded by connective tissue fibres of longitudinal direction. Numerous rounded cells adhere to the inside of the muscle fibres without forming a continuous layer. The three pairs of ostial orifices piercing the lateral heart wall are situated in the 3rd, 4th and 5th thoracic segments. In front and backwards the heart-tube continues with the anterior and posterior aorta respectively (Fig. 1A).

Four pairs of arteries arise from the heart: the first, running in antero-lateral direction, starts near the origin of the anterior aorta, while three others originate on the ventral side of the heart in the 4th, 5th and 6th segments. The walls of the arteries are very thin and not often noticeable, but their valves sometimes stain more deeply than the muscles of the heart, and then stand out distinctly. Even the play of these valves opening at each systole can occasionally be seen. Klövekorn (1934), who found these vessels in *Gammarus pulex*, called them 'Darm-Leber-Arterien', since they run to the digestive organs. It can be added that in methylene-blue preparations some finer branches of these vessels can be seen to ramify in the pericardial septum.

The heart is suspended in the pericardial cavity by means of bands of connective tissue, the strongest of them stretching from the terga to the dorsal

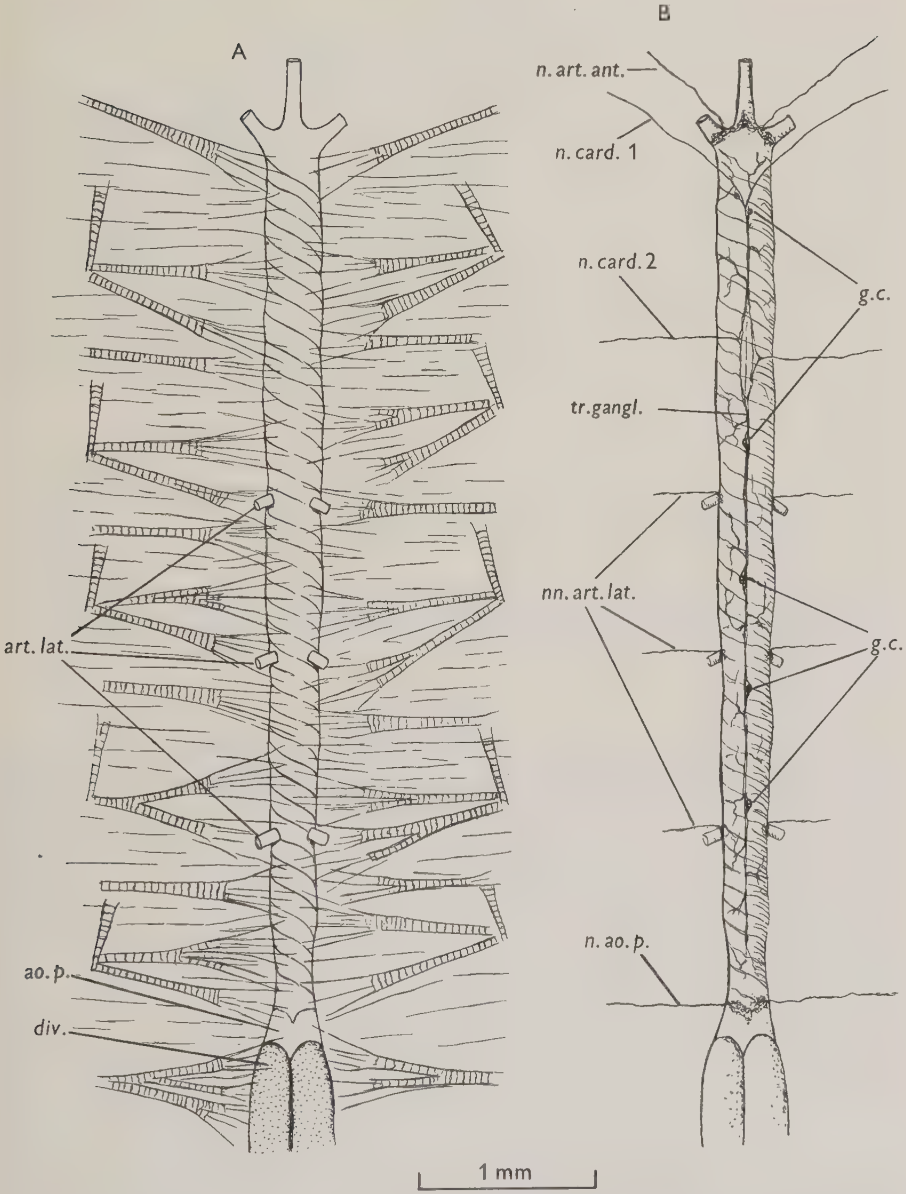

Fig. 1. *Marinogammarus marinus*. A: view of the heart from the ventral side with its arteries and alary muscles of the pericardium. *art.lat.* lateral arteries of the 4th–6th thoracic segments; *ao.p.* posterior aorta; *div.* coecal diverticula. B: view of the heart from the dorsal side. *tr.gangl.* ganglionic trunk; *g.c.* nerve cells; *n.card.*1, first cardiac nerve; *n.card.*2, second cardiac nerve; *n.art.ant.* nerve of arterial valves at the anterior end of the heart; *nn.art.lat.* nerves of the valves of the lateral arteries; *n.ao.p.* nerve of the valve of the posterior aorta. The ostia, situated on the lateral wall of the heart, cannot be seen in both figures.

side of the heart. The ventral side of the heart lies on and is connected with the pericardial diaphragm (called also pericardial septum).

Methylene-blue staining reveals in this membrane the presence of muscles which may be called alary muscles, as in insects, although their arrangement is not identical. While in insects, as well as in some crustaceans (Stomatopoda, Isopoda), they have a shape of fairly symmetrical triangles, they appear in *Marinogammarus* as ribbon-like bands, three or four in each half segment, arranged as shown in Fig. 1A. Similar muscle bands are also present in the abdominal segments in the horizontal septum which is the continuation of the pericardial diaphragm in this region. Klövekorn (1934), among many other details of the anatomy of the heart in *Gammarus pulex*, gives also a description of the alary muscles, but in his picture they appear to have a somewhat different disposition from that observed in *Marinogammarus*.

NERVES OF THE HEART

The elements of the heart innervation can be separated as (i) the local system, (ii) the nerves connecting the local system with the central nervous system, and (iii) the nerves of the arterial valves. There are, moreover, two systems of nerves which, although not directly connected with the heart itself, take part in the regulation of the blood circulation, viz. the nerves of the pericardial muscles and the nerves spreading over the pericardial wall.

Local System

The local system is made up of neurons with their cell bodies lying in a trunk, which may be called the main or ganglionic trunk, situated on the dorsal wall of the heart (Fig. 1B). The cells are of oval shape and measure approximately $15 \times 25\mu$. They are situated at a distance from each other, except that the two anterior cells may lie near together at the bifurcation of the trunk. The exact number of these cells, most probably 5 or 6, could not be established with certainty owing to the difficulties in staining them. For the same reason I am unable to state whether they are unipolar or bipolar, as they usually appear to be, or whether they have more processes.

The ganglionic trunk running in the middle line of the heart through nearly all its length, appears as a bundle of tightly collected fibres for the major part of its course. In the 3rd segment only, where it is joined by the second pair of cardiac nerves, its fibres frequently take a deviating course on one or both sides, and a bifurcation at the anterior end marks the point of entering of the first pair of the cardiac nerves. Most of the fibres of the trunk are the axons of its own nerve cells. They give off branches which, with their further divisions, supply the muscle fibres of the heart including those of the ostia but not those of the arterial valves. The finer ramifications are difficult to stain, but whenever the reaction is more favourable they appear, as in other crustaceans, abundantly distributed among the muscle fibres.

Nerves Connecting the Local System with the Central Nervous System

Two paired nerves which may be called the first and second cardiac nerves run from the neural cord to the ganglionic trunk of the heart (Fig. 1B, *n.card.* 1, *n.card.* 2). The fibres of the first of them are conveyed by a trunk emerging from the posterior part of the infraoesophageal ganglion (Fig. 2, *n.card.* 1). Not far from its origin it crosses a nerve trunk arising more anteriorly from the same ganglion and carrying fibres to the pericardial muscles and pericardial organs, and associates with it for a short distance (Fig. 2, *X*). As, besides, there are in this region anastomoses between various nerves, it cannot be excluded that in the cardiac nerve fibres of some other origin may also be present. From the point of junction mentioned above this nerve takes a more superficial course crossing the extensor muscles on their outer side near to their insertions. It then crosses on its inner side a thin oblique muscle (Fig. 2, *m*), inserting into the integument above the anterior end of the heart, and gives off a branch spreading over this muscle in a dense plexus. Passing on to the dorsal side of the heart the 1st cardiac nerve forms a Y-shaped figure with its fellow from the opposite side and with the ganglionic trunk.

The second cardiac nerve (Fig. 2, *n.card.* 2) runs to the heart with the dorsal branch of the segmental nerve originating from the connective linking the 1st and 2nd free thoracic ganglia (Fig. 2, *n.segm.*).This branch is a mixed nerve containing motor fibres passing to the dorsal layer of the extensor muscles, sensory fibres coming from the integument, fibres of the muscle receptors, and fibres to the pericardial organs (Fig. 2, on the right). The nerve running to the heart separates from other fibres at the lateral border of the median portion of the extensor muscles and passes on to the dorsal heart surface. It enters, often asymmetrically to that of the opposite side, the ganglionic trunk or one of its diverging branches, and bifurcates into branches running into opposite directions (Fig. 1B).

Each of the two cardiac nerves appears to consist of more than one fibre, but it is very difficult to ascertain their true number. Neither has it been possible to trace their course in the ganglionic trunk for a longer distance and find out their terminations. It may be assumed, with a great degree of probability, that they enter into relation with the cells of the local system.

Nerves of the Arterial Valves (Fig. 1B)

The nerves supplying the muscles of the arterial valves have no connexion with the elements of the systems described above. Two pairs of these valve nerves, viz. running to the anterior and posterior end of the heart, can be more easily noticed. The first of them approach the heart obliquely from the sides and, to judge from the direction of their course, come from the infraoesophageal ganglion, but could not be traced up to their origin. On reaching the valves the nerve fibres break up in a characteristic pattern of ramifications

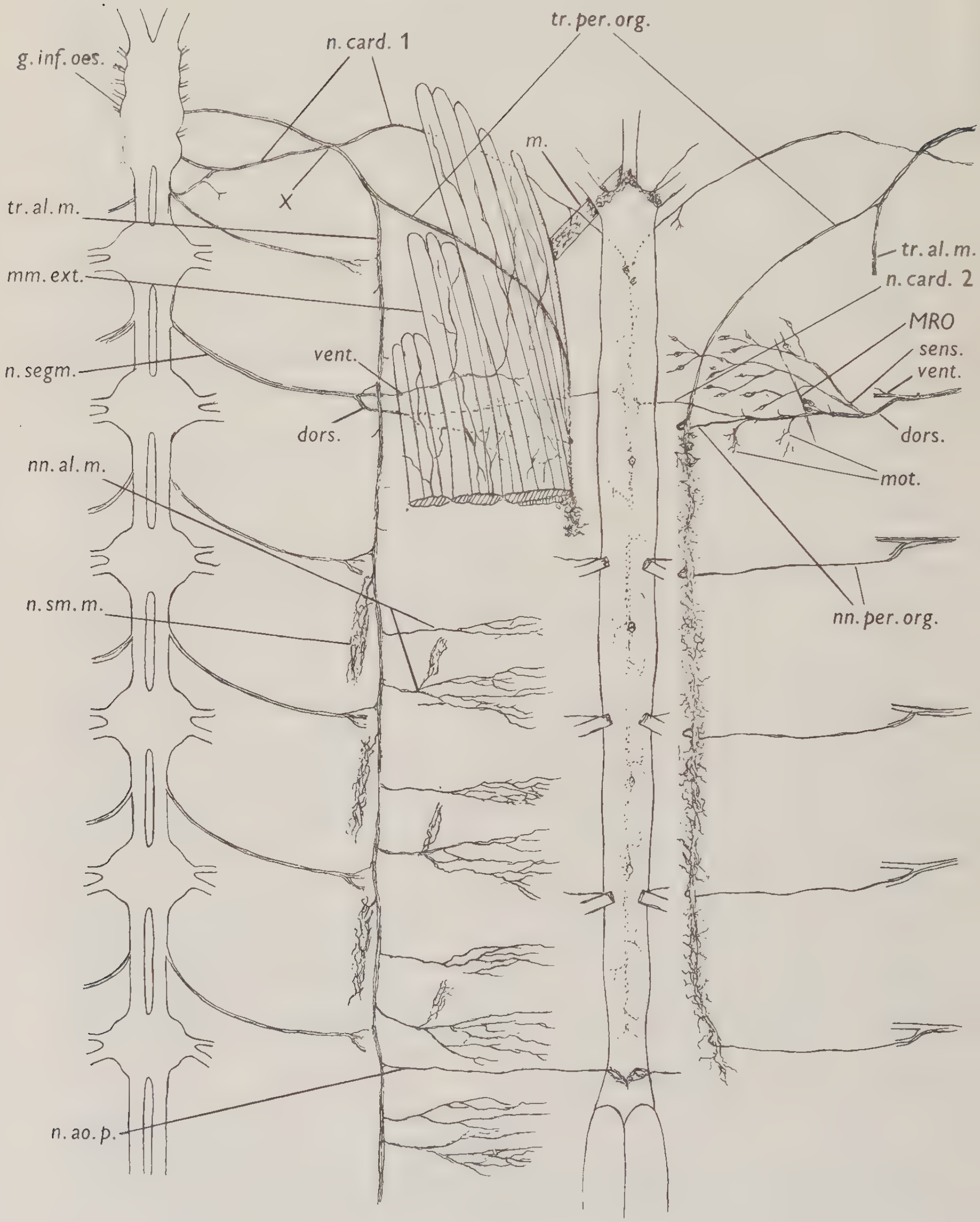
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Fig. 2. See opposite page.

different from that observed on the heart muscles. Some branches pass on to the valve of the anterior aorta and probably are the only source of innervation of this valve, since no separate nerve could be noticed to run down the aorta.

The nerves of the valve of the posterior aorta run transversely on the ventral surface of the extensor muscles. They arise from the longitudinal trunk giving off fibres supplying the alary muscles of the pericardium (Fig. 2, *n.ao.p.*), but, as pointed out below, this does not necessarily imply that they have a common origin and are in functional relation with this system of nerves.

The nerves of the valves of the arteries in the 4th–6th segments are not easily noticeable. Tiny filaments of transverse direction pass on to these valves, but do not stain clearly. Moreover, very fine connective tissue fibres running also transversely in the pericardial septum and looking often very similar to the fine nerves, contribute to the difficulties of interpretation.

Nerves of the Alary Muscles

The nerves of the alary muscles of the pericardium are given off by a trunk running on each side through the thorax and the abdomen at the lateral edge of the extensor muscles (Fig. 2, *tr.al.m.*). This trunk takes origin in the infraoesophageal ganglion, being in the first part of its course united into a common bundle with the trunk of the pericardial organs, as well as with some fibres supplying the ordinary muscles. When crossing the successive segmental nerves the trunk of the alary muscles is joined by fibres branching from these nerves, but whether these additional fibres take part in the innervation of the alary muscles, or travel in the trunk for a short distance only to leave it and supply the ordinary muscles, I cannot say. The branches innervating the alary muscles (Fig. 2, *nn.al.m.*) are of very fine calibre. There seem to be more

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Fig. 2. *Marinogammarus marinus.* Nerve elements controlling the blood circulation as seen in an animal sectioned along the left side and opened so that the neural cord is viewed from the dorsal side and the heart from its ventral side (semi-diagrammatic). The nerves represented in one plane of the drawing are situated in the animal in various planes and those running transversely follow in their course the curvature of the body; the nerves of the dorsal heart wall, same as in Fig. 1B, but seen here by transparency are drawn in dotted lines. *mm.ext.* extensor muscles sectioned at the level of the 3rd thoracic segment (in numbering the segments the first of thoracic segments is considered as fused with the cephalothorax); *m.* small oblique muscle; *g.inf.oes.* infraoesophageal ganglion; *n.card.* 1, 1st cardiac nerve; *X*, anastomose between the first cardiac nerve and the trunk bifurcating into the trunk of the alary muscles (*tr.al.m.*) and the trunk of the pericardial organs (*tr.per.org.*); *n.segm.* nerve arising from the connective and running to the dorsal part of the 3rd thoracic segment; *vent.*, *dors.*, its ventral and dorsal branches; on the left side of the figure the ventral branch is seen ramifying on the ventral side of the extensor muscles; on the right side the dorsal branch is shown conveying: sensory nerves (*sens.*), motor nerves to the superficial layer of the extensor muscles (*mot.*), second cardiac nerve (*n.card.* 2), nerve to the pericardial organs (*nn.per.org.*), and nerves of the muscle receptor organs (*MRO*); *nn.al.m.* nerves of the alary muscles; *n.sm.m.* nerve of small muscles at the lateral edge of the pericardial diaphragm; *n.ao.p.* nerve of the valve of the posterior aorta.

than one of them in each half segment, but they do not stain well and even in good preparations are difficult to detect.

From the same trunk arise also branches supplying small segmentally arranged muscles lying close to the trunk (Fig. 2, *n.sm.m.*). These muscle slips of longitudinal and slightly oblique direction are situated at the lateral edge of the extensor muscles, but appear as independent muscle units. As pointed out below, they may possibly be in some functional relation to the pericardial septum.

Nerves of the Pericardial Wall (Pericardial Organs)

The nerve trunk of the pericardial organs, after separating from the trunk of the alary muscles, crosses the ventral surface of the extensor muscles obliquely, and in the region of the 3rd thoracic segment passes on to their median surface (Fig. 2, *tr.per.org.*). In this position it runs backwards as far as the 7th segment. As the median surface of the extensor muscles constitutes the lateral wall of the pericardial cavity, this trunk comes to lie on the pericardial wall for most of its course. During this course it becomes strengthened by fine fibres which arise from the dorsal branches of the segmental nerves and curve ventrally round the convexity of the extensor muscles to join the pericardial trunks (Fig. 2, *nn.per.org.*). The first of them is situated in the 3rd segment and that can be more easily noticed. Their probable number is five on each side, but owing to their situation, particularly unfavourable both for staining and observation, this number is not certain.

There is a clear difference in structure of the trunks of the pericardial organs and that of the ordinary nerves, and even between the portions of the pericardial trunks themselves. Before passing on to the pericardial wall they look quite like the ordinary nerves, and so do the branches coming from the segmental nerves. On the pericardial wall, however, the trunks become broader, show uneven outlines, and look as if they consist of very small granules. Closer scrutiny of the best preparations reveals that the nerve fibres here break up into very fine filaments and during the staining process these in turn disintegrate into granules. In most favourable cases strands of such fine neuropile-like nerve tissue may be seen branching from the main trunks and entangling in a plexus spreading over the lateral wall of the pericardium. The more frequently observed uneven appearance of the trunk outlines is due to the incomplete staining of the strands starting from the trunks. Occasionally some bodies can be noticed which might be interpreted as indistinctly stained nerve cells, but their nature has always been doubtful.

Owing to the size of the animals and to technical difficulties in obtaining nerve preparations the picture of the nerves in the pericardium is not so impressive as in large crustaceans, but there can hardly be any doubt that these elements belong to the same category as the pericardial organs of the Stomatopoda and Decapoda described previously (Alexandrowicz, 1953*a*, *b*).

Discussion

The innervation of the heart of *Marinogammarus* proves to have much in common with that of other Crustacea and in particular, owing to the shape and anatomy of the heart, with that of the Isopoda (Alexandrowicz, 1952). There is, however, a difference in the situation of the ganglionic trunk, in that in the Isopoda investigated (*Ligia oceanica*, *Mesidotea entomon*, *Idotea emarginata*) it lies on the inner surface of the heart, while in the Gammaridae it lies outside of it. The latter position is the same as in the Stomatopoda (Alexandrowicz, 1934), and, according to some observations I have recently made, in the Mysidacea too. Otherwise the ganglionic trunk appears to have a similar shape and structure as in isopods, i.e. it is composed of a few neurons of the local system and some fibres connecting this system with the neural cord. As in other crustaceans the local system should be regarded as an autonomous apparatus ruling the heart beat.

The situation of the heart in the Amphipoda extending up to the cephalothorax proved to be more favourable than in the Isopoda for the observation of the nerves connecting the heart with the neural cord, and gave the chance of establishing the origin of these nerves as from the infraoesophageal ganglion and the 1st free thoracic ganglion. There are, however, still some doubtful points. For instance, as the 1st thoracic segment is fused with the cephalothorax and its ganglion with the infraoesophageal ganglionic mass, the question remains whether the 1st cardiac nerve originates in the ganglion of the 1st thoracic segment, which judging from its course is more likely, or from one of the cephalic segments. If the former is true, the cardiac nerves, which presumably have a regulatory function on the heart rhythm, would originate in the two anterior thoracic segments (the term segment being used as equivalent to metameres).

As regards the innervation of the arterial valves a particular feature in gammarids would be the absence of the nerve running down the anterior aorta, which, known as nerve of Lemoine, was one time considered to be the main nerve of the heart of decapods, but was found in these animals as well as in stomatopods and isopods to supply only the valve of the anterior aorta (Alexandrowicz, 1932, 1934, 1952). In gammarids, unless this negative evidence is due to some particularly refractory behaviour of this nerve to staining with methylene blue, it appears to be wanting.

The question whether the innervation of the arterial valves is linked with that of the pericardial muscles in a way securing their synchronous action remains open. In decapods all these nerves, with exception of that supplying the valve of the anterior aorta, arise from a common bundle, whereas in stomatopods the two systems have independent courses; in isopods their relations have not been determined, and the fact that in *Marinogammarus* the nerve to the posterior aorta arises from the trunk of the alary muscles does not prove that the fibres of both have a common origin.

The assumption that the nerves of the valves and of the alary muscles are in functional relation is insecure until the role of the pericardial muscles is definitely established. The opinions of the writers who have studied this problem in crustaceans as well as in insects differ as to whether the function of these muscles may be passive or active, i.e. whether they simply strengthen the elastic resistance of the pericardial septum, or whether their contractions contribute to the movements of this septum. Even the two authors who described the alary muscles in amphipods, v. Haffner (1934) in *Phronima sedentaria*, and Klövekorn (1934) in *Gammarus pulex*, are not quite in accord. On this question the following remarks may be added. First, it seems rather unlikely that organs made up of cross-striated muscle tissue and supplied by a special system of nerves would function merely as elastic bands; and, secondly, that considering their possible action there should be taken into account not only their relation to the heart-beat, but their possible co-ordination with the movements of the animal. It is obvious that during the movements of the body the respective pressure in the large sinuses can vary widely and if, as is likely, such fluctuations of the blood pressure in the pericardial cavity would impair the heart action, the appropriate contraction of the alary muscles might regulate the position of the pericardial diaphragm and compensate for these changes. It is, moreover, possible that not only the alary muscles, but also those small muscle slips mentioned above as receiving their nerve supply from the trunk of the alary muscles, are active in the same way. They end, in fact, exactly at the level of the lateral attachment of the pericardial septum and it appears very likely that their contractions may have a pulling action on the latter. It should be mentioned that in *Phronima* v. Haffner has found small muscles attached to the lateral edge of the pericardium which, as he puts it, 'dienen...zur Befestigung des Pericardialseptums'.

The nerves expanding over the pericardial wall in neuropile-like plexuses show such a resemblance in their mode of termination to those found in stomatopods and decapods that their inclusion in that category of elements called pericardial organs may be considered as well founded. It is therefore highly probable that they have a neurosecretory function, releasing into pericardial cavity some hormone or hormones which influence the heart rhythm in a similar way as in those animals (Alexandrowicz & Carlisle, 1953). In the present description of these organs it has been pointed out that no uncontestable evidence of the presence of nerve cells in them could be obtained. If the assumption that they are missing is correct, all the fibres of the pericardial trunks would come from the cells situated in the central nervous system, and thus this arrangement in gammarids would resemble more that in decapods than in stomatopods, since in the latter most of the fibres of the pericardial organs originate in the nerve cells lying outside the ganglionic cord.

It is interesting to note that different elements of the systems described above, viz. the first of the cardiac nerves, the trunk of the alary muscles, and the trunk of the pericardial organs, originate in the infraoesophageal ganglion. Hence it may be conjectured that the centre controlling the blood circulation could be localized in this part of the nervous system. Admittedly some of the nerves described, as the 2nd cardiac nerve and the additional nerves to the pericardial organs, start from the thoracic ganglia, but it is possible that the cell-bodies of their neurons are situated in the infraoesophageal ganglion while their axons run down the neural cord to leave it with the nerves arising farther backwards. This problem must await solution until by successful tracing of all the nerves in question their true origin and the connexions of their neurons can be established.

SUMMARY

In the heart of *Marinogammarus marinus* three systems of nerve elements have been found, viz. (1) a local system consisting of a ganglionic trunk situated on the dorsal surface of the heart and sending branches to the muscle fibres, (2) two paired nerves, termed the 1st and 2nd cardiac nerves, connecting the local system with the central nervous system, and (3) nerves supplying the muscles of the arterial valves. It is assumed that the local system rules the beat of the heart and that the cardiac nerves have a regulating function.

Not directly connected with the heart, but having relation to the blood circulation are two sets of nerves: (1) nerves supplying the alary muscles, presumably regulating the pressure in the pericardial cavity, and (2) nerves spreading over the lateral wall of the pericardium in neuropile-like plexuses and supposed to have a neurosecretory function.

It is suggested that the centre controlling the blood circulation could be localized in the infraoesophageal ganglion.

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J. Mar. biol. Ass. U.K. (1954) 33, 721–732
Printed in Great Britain

A MARINE FUNGUS *PLECTOSPIRA DUBIA* N.SP. [SAPROLEGNIACEAE], INFECTING CRUSTACEAN EGGS AND SMALL CRUSTACEA

By D. Atkins, D.Sc.

(Text-figs. 1–5)

Among eggs, cut from a recently dead *Pinnotheres pisum* on 11 June 1953 (spawned on 9 May), about six were infected with a fungus *Plectospira dubia* n.sp. not previously observed and quite distinct from both *Leptolegnia marina* (Atkins, 1929, 1954*a*) and *Pythium thalassium* (Atkins, 1954*b*) also parasitic in crustacean eggs. The parent showed no fungus in the gills (the body was not examined). The crab had been in Plymouth laboratory tank water of salinity of 36–37‰ with added townettings from 26 August 1952 to the time of death, for 3 months of the time within a *Mytilus edulis*. The eggs were kept in a finger bowl, as it was hoped they would hatch, but in little more than a week most of the eggs of the small egg-mass were infected. This occurred with temperatures between 14 and 15° C.

The same fungus developed independently in the eggs taken, in clean and healthy condition, from a recently dead *Gonoplax rhomboides* which had been in the laboratory for some time. This infection started in the form of a mycelium given over entirely to the production of gemmae; but mycelia giving rise to sporangia developed in other eggs of this egg-mass within a few days (see also p. 728).

The following crustacean eggs became infected by placing one to several infected eggs with them: *Typton spongicola*, *Crangon vulgaris*, *Leander serratus*, *Macropodia* sp. and *Portunus depurator*. All infected eggs had embryos in various stages of development, up to about two-thirds developed.

The fungus is of dense, but slow, growth. The first sign of infection in the egg was the development of a space between the inner and outer egg membranes, and in this space the young infecting hyphae first appeared (Fig. 1D). The space increased with the growth of the infection and this, together with some bulging of the sporangia beyond the egg, increased the size of infected eggs. The healthy egg of *Pinnotheres pisum* is about 0·33 mm in diameter, that of the infected egg about 0·5 mm. The egg of *Gonoplax rhomboides* increased with infection from the normal diameter of about 0·5 mm to 0·65 mm; that of *Portunus depurator* from about 0·32 mm to 0·42 mm; and that of *Crangon vulgaris* from 0·8 by 0·58 mm to 1·3 by 0·8 mm. The eggs of *Gonoplax rhomboides* with the gemmae stage of the fungus did not show increase in size.

Plectospira dubia while an active parasite—infecting hyphae entering eggs containing embryos with heart beating—does not appear to be as severely pathogenic as *Leptolegnia marina* (Atkins, 1929, 1954*a*). The hyphae seem to be first attracted toward the yolk remnant, but a few prezoeae and zoeae have become infected and also three *Evadne*: it is possible that the latter may have been moribund before infection.

The method of sprouting of the infecting spores has not been clearly seen, as they tend to collect in groups, which soon become coated with other organisms.

Mycelial characteristics

The hyphae are generally stout; the mycelium becomes irregularly branched; the ends of branches tend to be swollen. Young hyphae soon after entry attain a width of about 27μ, and the main hyphae of a young mycelium may be 50μ (Fig. 1C). The cytoplasm is hyaline, but with groups of minute shining granules, the groups more or less evenly spaced, as though collected round nuclei (Fig. 1A, B, D).

Asexual reproduction

Within the host the growth soon becomes irregular (Fig. 1A), the branches inflated and bulbous, up to about 100μ wide, acting as sporangia. So far as can be seen in such a restricted space there is no distinction between vegetative and reproductive hyphae, and eventually the whole of the mycelium is used up in spore-formation and the host is left a network of empty sporangia (Fig. 2A).

The inflated sporangia for the most part are within the host tissue, but characteristically bulge beyond the crustacean egg (Fig. 2B, C); occasionally much of a sporangium may be extra-matrical. In a covered dish from which there had been some slight evaporation with increased salinity the extra-matrical portions of sporangia were exceptionally inflated (Fig. 2D). The inflated sporangia with large central vacuole and widely spaced spore initials gives to an infected egg a coarsely vesicular appearance.

Sporangia are provided with one or more efferent hyphae, $50–400\mu$ long, and $10–30\mu$ wide (Fig. 3). When long the tubes are frequently corrugated and may be twisted (Fig. 3A). Sporangia quite often have paired efferent hyphae (Fig. 3D).

Spores are formed not only in the sporangia, but in the efferent hyphae, except for an apical portion of a length up to about 50μ, which remains hyaline. During spore formation the tip of the efferent hypha frequently enlarges and becomes flattened across the top and when the tip dissolves or gives way—it is not known which occurs—the aperture is then left with flared lips. Other shapes of aperture are shown in Fig. 3C. Occasionally the pore may be exceedingly small, so that zoospores in the second motile stage,

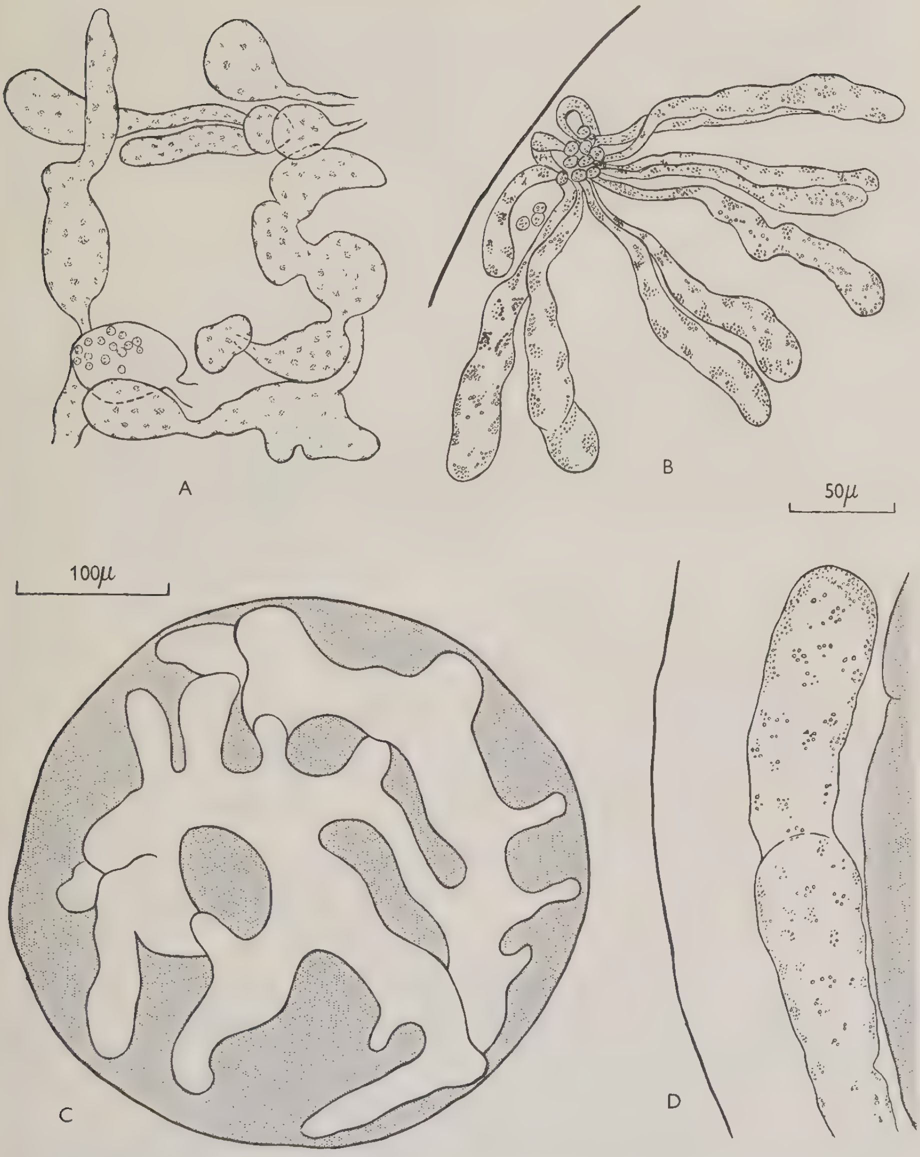

Fig. 1. *Plectospira dubia.* A, mycelium dissected from egg of *Macropodia*; B, group of young infecting hyphae below dorsal cuticle of *Evadne*; C, young mycelium in egg of *Pinnotheres pisum*; D, young hypha growing in space between outer egg membrane and embryo.

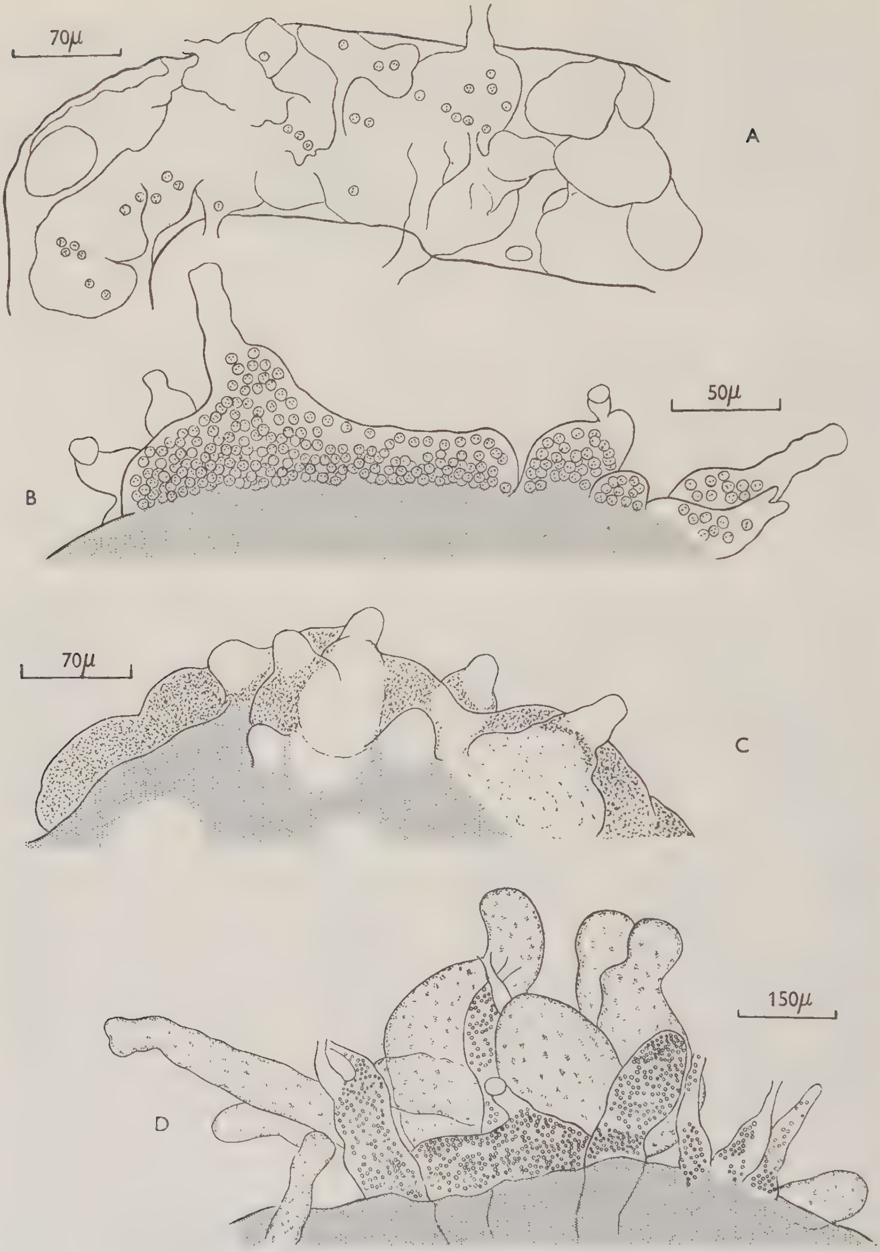

Fig. 2. *Plectospira dubia.* A, sporangia, empty except for a few encysted spores, within abdomen of a zoea; B, sporangia, containing encysted spores, projecting from egg of *Pinnotheres pisum*; C, young sporangia, with large vacuoles, projecting from egg of *P. pisum*: the growing efferent hyphae have clear tips; D, much inflated sporangia projecting from egg of *Crangon vulgaris*: taken from a dish in which there had been some increase in salinity.

in passing through are constricted and have a difficult and slow passage: one was observed to be pinched almost in two, the portion beyond the aperture rounding up as did the larger portion inside, so blocking the aperture. The tips of all the external hyphae of a sporangium do not always give way, as occurs also in *Plectospira myriandra* and *P. gemmifera* (Drechsler, 1927, 1929).

Pyriform flagellated zoospores are formed within the sporangium and efferent hyphae.

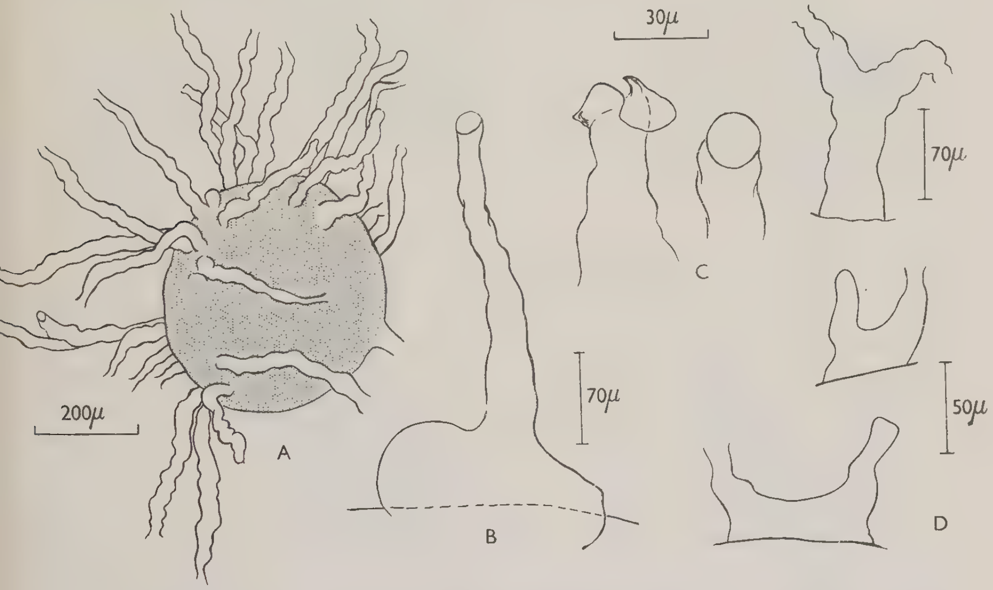

Fig. 3. *Plectospira dubia.* A, corrugated efferent hyphae on egg of *Pinnotheres pisum*; B, external portion of sporangium with a single efferent hypha; C, apertures of efferent hyphae. D, three external portions of sporangia with paired efferent hyphae, two of which have not functioned.

Only about nine instances of zoospore formation have been watched, for it occurs mostly at night, rarely during the day. Spore initials (Fig. 5*c*) are visible for some hours, for as many as five, before moving zoospores are formed. The active formation of zoospores is generally preceded by contraction of the contents of the sporangium. The degree of contraction varies, the contents rapidly withdrawing entirely from the efferent hyphae, and in extreme instances contracting from the wall of the sporangium. It would seem that contraction is greater if it occurs when the spore initials are not widely separated. In one sporangium, watched between 4 and 9.20 p.m. G.M.T. (Fig. 4), the sudden contraction was so great that the appearance of spore initials was lost, and the contents of the sporangium appeared darkly granular with a lighter edge. There followed a gradual expansion, small rounded

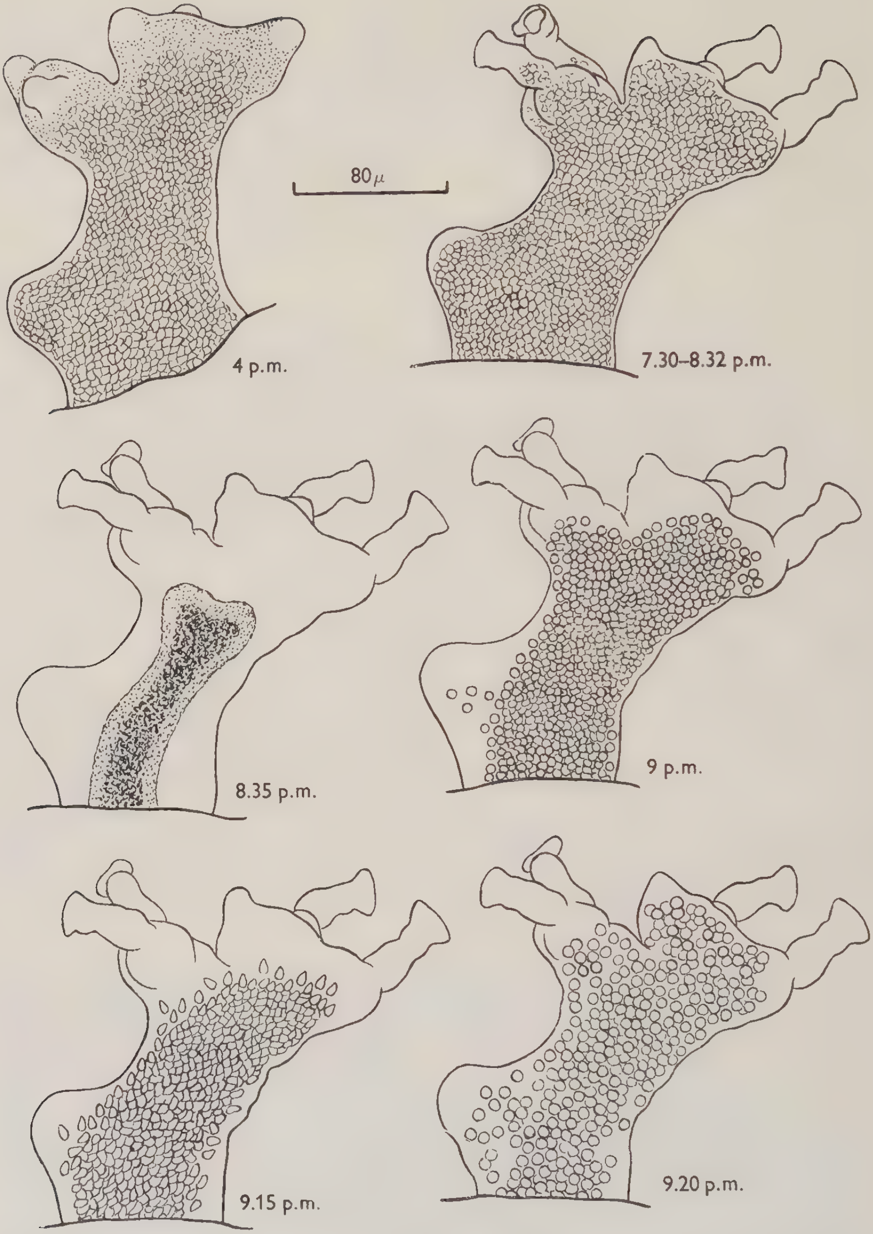
80μ
4 p.m.
7.30–8.32 p.m.
8.35 p.m.
9 p.m.
9.15 p.m.
9.20 p.m.

Fig. 4. *Plectospira dubia.* Formation of zoospores in external portion of a sporangium: for description see text pp. 725–7.

bodies appearing on the edge of the protoplasmic mass. Expansion continued and rounded spores became clearly visible; these gradually became pear-shaped, and the appearance was then of a moving and heaving mass of spores, which were evidently flagellated. On the outskirts of the main body single zoospores rocked gently. A high-power objective was lowered into the water to get a clearer view, but unfortunately it had an adverse effect, for the spores rapidly rounded up within the sporangium.

In another sporangium kept under observation at the same time, in a separate watch-glass, the spore initials were widely spaced before contraction, and although the contents withdrew from the two efferent hyphae of a length of about 150μ, there was little contraction in the sporangium itself: the spore initials remained visible. On expansion, the spores reached about half-way along the efferent hyphae. Moving zoospores were visible, but although a few spores moved to near the end of the efferent hyphae, none escaped; all encysted.

One day, from about 5 p.m. G.M.T., four efferent hyphae were kept under observation. In three the process of spore formation was as described, with moderate degree of contraction and expansion of contents of the sporangium and all spores encysted; none escaped. In the fourth, on expansion, a single string of zoospores, diamond-shaped and attached end to end by delicate strands of protoplasm, passed up the efferent hypha. At intervals eight feebly rocking pyriform zoospores slowly emerged from the aperture, swam away slowly at first, then with more speed. No doubt these zoospores were biflagellate, as usual in many members of the Saprolegniacea, but it was not possible to determine this, as it had been found that lowering a high-power objective into the water caused encystment. The remaining spores encysted in the sporangium and did not begin to emerge from the efferent hypha in the second motile stage until about 8 p.m. G.M.T. next day.

It is apparently during the formation of the pyriform zoospores that the tips of the efferent hyphae give way, but it is not always possible to be sure that a pore is actually present unless zoospores escape.

The pyriform zoospores are only feebly active within the sporangium, and that usually for not more than 5–10 min, or less. The time they remain encysted varies: sporangia left with newly encysted spores overnight may be found with empty cysts the next morning, or spores may still be present 20 hours later.

The fact that many crustacean eggs contained mycelia crowded with encysted spores confirms that the behaviour of the pyriform zoospores is as observed in the few instances recorded.

Zoospores in the first motile stage are about 10μ long and when encysted are 7–8μ in diameter.

Owing to the large central vacuole of the sporangium, the generally wide spacing of the spore initials and the length of clear protoplasm at the end of the efferent hyphae, there is sufficient space for at least some of the pyriform zoospores to round up; these seem to collect round the walls, leaving a central space through which the more internally situated zoospores can pass on their way to the exterior. When crowded the encysted spores are angular.

Zoospores have not been seen actually emerging from cysts within the sporangium, but were seen escaping singly at intervals through the apertures of the efferent hyphae at all times of the day. They are rather long and thin, distinctly grooved longitudinally, with two long, laterally inserted, flagella, one directed anteriorly and one posteriorly: the flagella work with a looping motion. Zoospores in the second motile stage are 11–12μ long: when encysted they are 6–7μ in diameter. Time normally spent in the second swimming stage is unknown. They have been seen to encyst within a few minutes of emerging from the sporangium, but this is unusual and probably due to unfavourable conditions on a slide. These zoospores swim actively within the sporangium and efferent hyphae seeking a way of escape, for the tips of all efferent hyphae do not give way; on emergence they swim rapidly away.

The walls of the sporangia and efferent hyphae are persistent and do not disintegrate or rupture irregularly as do the sporangia of *Thaustotheca* and some species of *Dictyuchus* for the liberation of the laterally biflagellate zoospores (Coker & Matthews, 1937, pp. 50, 52).

The thin-walled empty cysts are visible in the sporangia and basal parts of efferent hyphae after the escape of the zoospores: they are round or angular according to the degree of crowding.

Proliferation

Very occasionally proliferation occurs, a new sporangium growing up within the old one (Fig. 5A, B), as occasionally in *Leptolegnia* and typically in *Saprolegnia*. The new growth may extend beyond the old (Fig. 5B), or may not reach the end of the old efferent hyphae, when it may send out short exit tubes; in the example figured there were eight (Fig. 5A).

Gemmae

The first appearance of *Plectospira dubia* in eggs (with embryos two-thirds developed) removed from a recently dead *Gonoplax rhomboides* occurred a week after their removal, and was under a form quite different from that previously seen: it was distinctive because of spherical bodies of varying size, showing white by reflected and dark by transmitted light. The plants under this form did not entirely fill the egg, and infected eggs were no larger than healthy ones, but the magenta colour from the small yolk remnant was diffused throughout the egg. Young plants dissected out from the egg had the appearance shown in Fig. 5D. The cytoplasm was finely and densely granular; the mycelium was producing rounded bodies up to about 100μ in diameter,

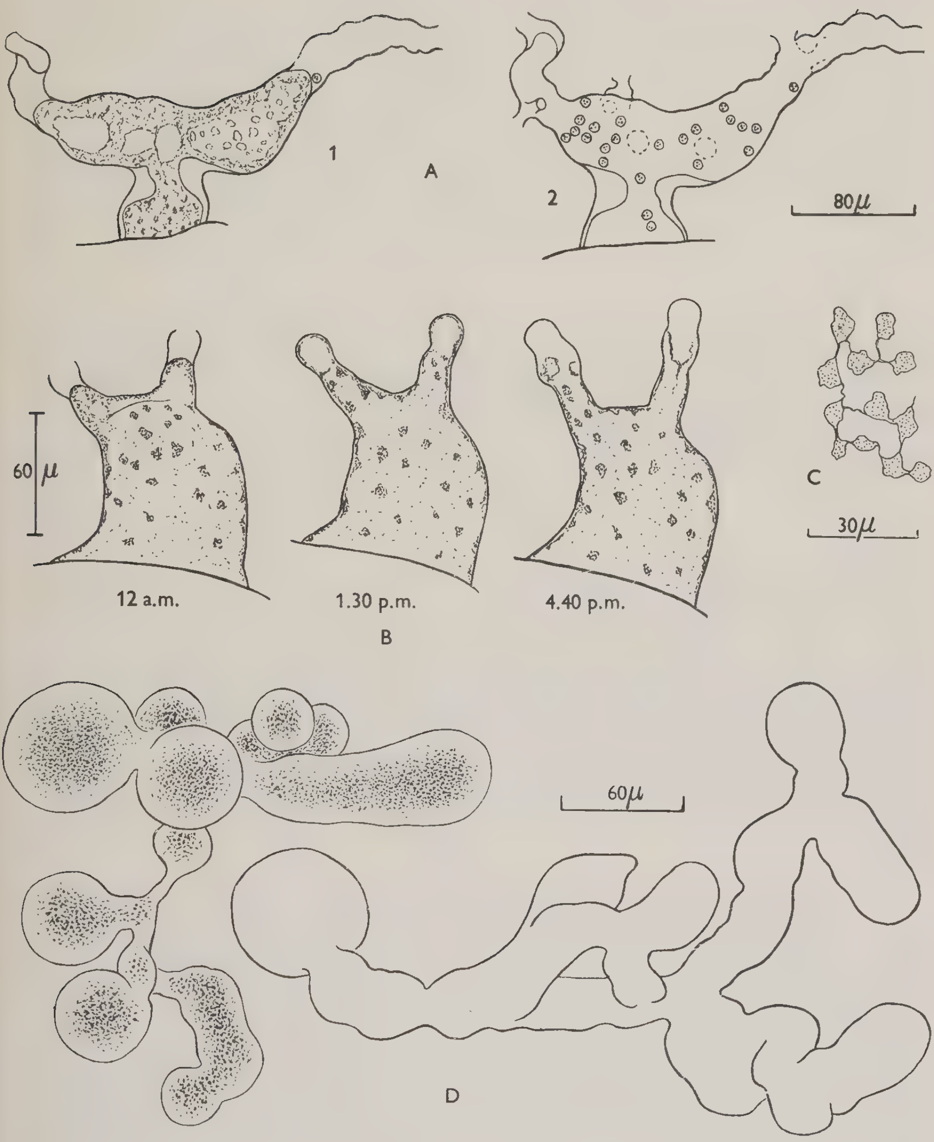

Fig. 5. *Plectospira dubia.* A and B, two instances of proliferation: in A 1 the protoplasm is much vacuolated; in A 2 the apertures of four efferent hyphae on the under surface are indicated by broken lines; C, spore initials in an efferent hypha. D, mycelia producing gemmae, dissected from egg of *Gonoplax rhomboides*: contents indicated in mycelium on left.

which became separated from the mycelium. When many gemmae were present, no obvious hyphae remained. Plants remained in this condition for periods of up to 2 weeks. The rounded bodies would appear to be a resting stage and, although they had thin walls, are evidently gemmae. Eventually they became vacuolated and turned into sporangia with efferent hyphae. Gemmae have not been seen on plants which had already produced sporangia, for the mycelium is entirely used up in zoospore formation: this may be due to the small size of plants in small hosts.

Sexual reproduction

This has not been seen. For over 3 months in 1953 the fungus was grown in crustacean eggs placed in small bowls of sea water of salinity 36–37‰; some bowls were partly immersed in running tank water which kept the temperature between 14 and 18° C, and others were kept at a constant temperature of 15–16° C: in none of the bowls did the fungus produce sexual organs during this time.

Taxonomy

The new fungus although clearly a member of the Saprolegniaceae is difficult to assign to any existing genus. It would seem to resemble *Plectospira* (Drechsler, 1927, 1929) in that the sporangia are composed of inflated elements with efferent hyphae, frequently of uneven diameter, but while in *Plectospira* there is a clear distinction between slender vegetative hyphae and swollen sporangia, the former never being converted directly into sporangia, in the new fungus, so far as can be seen under crowded conditions in crustacean eggs and small Crustacea, there is no distinction between vegetative and reproductive hyphae, the whole mycelium being used up in zoospore formation.

The sporangial contents of *Plectospira* behave as in *Achlya* and *Aphanomyces*, encysting in a cluster immediately on leaving the sporangium (Drechsler, 1927, 1929); those of the new fungus do not. Although a few of the first zoospores may escape from the sporangium after the manner of *Saprolegnia* and *Leptolegnia*, most of them after a short period of feeble activity within the sporangium encyst there, and later leave their cysts and escape by the apertures of the efferent hyphae. The sporangia would seem to be of the false-net type sometimes occurring in *Achlya*, in that the sporangial wall persists (Couch, 1931, p. 225). In *Dictyuchus*, although the first zoospores encyst within the sporangium, the secondary ones either escape from their cysts directly through the wall of the sporangium, or else in the false-net group the wall of the sporangium quickly disappears.

In both *Saprolegnia* and *Achlya*, according to Coker (1923, p. 9), 'it frequently happens that the discharge of the spores is only partial, a few or even a good many spores being left in the sporangium. These retained spores may emerge from their cysts, as normally, for a second swimming stage, moving about within the sporangium until they find their way out by its mouth, if they ever do.' This partial retention of the pyriform zoospores in *Saprolegnia* and *Achlya* is carried much further in the new fungus and is the normal occurrence; moreover the latter differs from both *Saprolegnia* and *Achlya* in that the sporangia have no definite shape, but are lobulate inflated segments.

Rather than create a new genus for this marine fungus, while the sexual organs are unknown, it is proposed to place it provisionally in the genus *Plectospira* with the specific name *dubia*.

Diagnosis

Mycelio plerumque intramatricali, rudi, composito de hyphis irregularibus latitudine (50–100 μ); sporangiis irregulariter inflatis, subinde proliferosis, depletis per hyphas efferentes, longitudine variabiles et saepe latitudine irregulares; sporangii pariete persistenti; zoosporis primas et secundas capsulas formantibus, primo tamen statu natanti plerumque suppresso; zoosporis plerumque capsulas formantibus intra sporangium postquam paulisper infirmiter nataverunt, postea valide natantibus ad secundum statum; sporis tum 6–8 μ latitudine cum in capsulas inclusa sunt; gemmis numerosis, sphaeralis, ad 100 μ latitudine; partibus sexualibus ignotis.

Mari incolens; parasitica in ovis crustacearum et in parvis crustaceis.

Type material has been deposited in the British Museum (Natural History).

This work was made possible by the kindness of the Board of Studies in Zoology of London University in granting me the use of the London University Table at the Laboratory of the Marine Biological Association at Plymouth, and was done while working on the biology of *Pinnotheres*. I am indebted to Dr B. Barnes for reading the manuscript. Dr J. Morton has most kindly turned the diagnosis into Latin.

SUMMARY

A marine fungus, *Plectospira dubia* n.sp., has been found infecting crustacean eggs and small Crustacea in the Plymouth Laboratory. It has the following characteristics: mycelium mostly intra-matrical, coarse, hyphae 50–100μ in diameter. Sporangia irregularly inflated, emptying by efferent hyphae of variable length and frequently of irregular diameter; sometimes proliferous; wall persistent. Zoospores dicystic and dimorphic, but the first swimming stage largely suppressed, the first zoospores mostly encysting within the sporangium after a short period of feeble activity, then swimming in the second stage. The first zoospores pyriform and flagellate (probably biflagellate): in the second motile stage laterally grooved with two laterally inserted flagella. First zoospores about 10μ long, when encysted 7–8μ in diameter; in the second motile stage 11–12μ long, when encysted 6–7μ in diameter. Gemmae numerous, spherical, up to 100μ in diameter. Sexual organs so far unknown.

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J. Mar. biol. Ass. U.K. (1954) 33, 733–738
Printed in Great Britain

BLOOD PERFUSION OF THE KIDNEY OF *LOPHIUS PISCATORIUS* L.

II. INFLUENCE OF PERFUSION PRESSURE ON URINE VOLUME

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(Text-figs. 1–4)

In a previous publication (Brull, Nizet & Verney, 1953) it was shown that the kidneys of *Lophius*, perfused with heparinized blood, secrete urine which is similar to bladder urine. One of the conclusions was that 'changes in perfusion pressure up to 100–150 mm of water, raised the urine flow; above 150 mm there was no effect'. The object of the experiments described below was to investigate the relationship between perfusion pressure and urine flow, especially below the critical level of 150 mm water.

The experiments were carried out in July–August in the Plymouth Laboratory, where we were provided with a large supply of live *Lophius*, for which we are much indebted to the Director and Staff of the Laboratory.

Methods

We improved our perfusion technique by the use of a pump made of plexiglass, and a small rotating oxygenator built by one of us (Y. Cuypers) for this purpose.

The pump and oxygenator were started working before the kidneys were connected, so as to oxygenate the blood. A closed circulation circuit was made with plastic tubes, from the reservoir to the kidneys and from the kidneys to the pump. To prevent twisting of the vessels, the kidneys were laid on a thin layer of perforated wood on to which the cannulae could be tied down.

The venous flow was measured directly with a burette and stop-watch. The experiments were carried out at room temperature.

Results

Experiment 9

Two kidneys, from the same *Lophius* weighing 4·8 kg, were perfused by the same pool of blood, kidney A at a pressure of 80 mm water, and kidney B at 260 mm throughout the experiment (Table I).

The blood flow in kidney B was much higher than in A; but the figure was missed by accident. In this pair of kidneys, the urine flow was no better at 260 mm than at 80 mm; we are very likely above the critical maximum level of perfusion. The secretion needs to be studied while starting at lower perfusion pressures.

TABLE I. PERFUSION EXPERIMENT 9

	Kidney A (weight 9·5 g)				Kidney B (weight 9·5 g)			
		Urine				Urine		
Time (min)	Blood flow (ml./g/min)	No.	Vol. (ml.)	Flow (ml./g/h)	Blood flow (ml./g/min)	No.	Vol. (ml.)	Flow (ml./g/h)
0	Perfusion started							
30	—	—	—	—	Perfusion started			
45	Pressure regulated at 80 mm				Pressure regulated at 260 mm			
71	—	First drop in 3 min			—	First drop in 3 min		
130	—	1	1·0	0·10	—	—	—	—
145	—	—	—	—	—	1	1·0	0·09
245	1·3	2	2·0	0·13	—	2	1·7	0·10

Both ureteral cannulae contain 0·5 ml. extra urine.

TABLE II. PERFUSION EXPERIMENT 13

	Kidney A				Kidney B			
Time (min)	Perfusion pressure (mm blood)	Blood flow (ml./g/min)	Urine no.	Urine flow (ml./g/h)	Perfusion pressure (mm blood)	Blood flow (ml./g/min)	Urine no.	Urine flow (ml./g/h)
0	80	Perfusion started						
15	—	—	First drop					
45	—	—	—	—	25	Perfusion started		
60	80	0·45	—	—	25	0·10	—	—
74	—	—	1	0·18	—	—	—	—
100	80→70	—	—	—	25→70	—	—	—
107	70	0·40	—	—	—	0·70	—	—
120	—	—	—	—	—	—	First drop	
140	—	—	2	0·16	—	—	—	—
145	70→45	—	—	—	—	—	—	—
150	—	0·30	—	—	—	—	—	—
200	45→75	0·24	3	0·12	—	—	1	0·80
295	75	—	4	0·11	70	—	2	0·10

Experiment 13

Perfusion with the same pool of blood was carried out on two kidneys: kidney A weighing 11 g, from a fish of 6·4 kg; kidney B weighing 6 g, from a fish of 3·5 kg (Table II). At the first three stages of perfusion pressure, 80, 70 and 45, the urine flow of kidney A is parallel to the pressure: 0·18, 0·16 and 0·12. After 3 h 20 min of perfusion, the kidney no longer responds to a rise of pressure from 45 up to 75. Perfusion of kidney B at the low pressure of 25 gives no urine; at 70 mm secretion starts with an average output of 0·09.

Exps. 9 and 13 confirm our previous results, namely that the kidney is sensitive to changes in pressure below about 100 mm of water. We therefore decided to investigate this in detail by recording the number of drops of urine as a function of perfusion pressure.

Experiments 21–23

In these three experiments (see Fig. 1) two kidneys from the same fish were simultaneously perfused by the same pool of blood at rising or decreasing pressures. The arrow on the tracings indicates whether the perfusion is started at low or high pressure. Urine drops were recorded and the urine flows are translated into ml per gram of kidney per minute.

At each rise of pressure the reaction of the kidney is usually slow, and a certain time may elapse before the urine flow reaches its maximum, as shown where some curves slope up at the beginning and down later when the pressure is lowered (22 B).

In Exp. 21, we succeeded in perfusing one kidney only; during the first 2 h of perfusion, the pressure was progressively lowered (see curve $21B_1$), while during the next 2 h, the perfusion was carried out at rising pressures ($21B_2$).

The most striking facts shown by the slope of the six curves would seem to be the following. (1) Below about 25–30 mm pressure, no urine is secreted. (2) Between 30 mm and about 200–250 mm, the volume of urine secreted per minute follows the perfusion pressure in a regular way, the response following an exponential curve. (3) Above perfusion pressures of about 200–250, the urine flow is no longer influenced by further rises of pressure; this last finding confirms the results of the first publication on the same subject (Brull *et al.*, 1953).

The *blood flow* through the kidneys is of the same magnitude as in the experiments previously published (Brull *et al.*, 1953), in which arterialized blood was used. The results are shown in Figs. 2–4. In kidney A, Exp. 23, and at low pressure, we found the curve had the same tendency as reported before to change its slope (Fig. 4).

Discussion

In our opinion, one striking fact appears from these results: below a critical perfusion pressure varying from about 100 up to 225 mm from one kidney to the other, the urine flow responds to the pressure in the form of an exponential curve. We have plotted the figures of urine flow against the blood flow, but have not reproduced them here, because these curves are similar to those shown in Fig. 1. It would be interesting to find out which of the two factors is the determining one: pressure or blood flow.

In Exp. 21 (Fig. 2), there are two perfusion phases: we started with a pressure of 190 mm, decreased it to 75 mm, and raised it again to 220 mm;

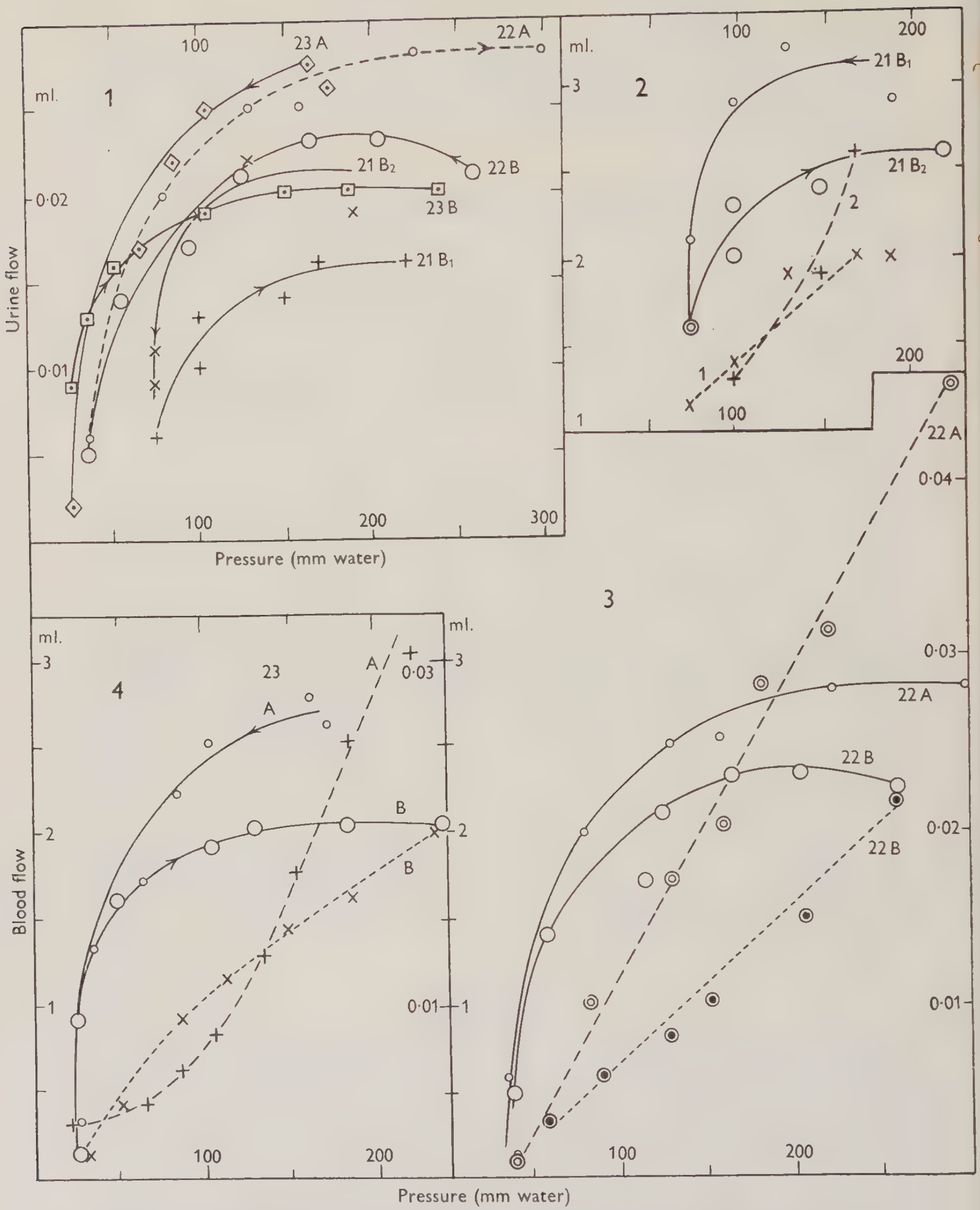

Fig. 1. Urine flow (in ml./g/min) plotted against perfusion pressure (in mm water) in five kidneys, two in Exp. 22, two in Exp. 23, one in Exp. 21 at two stages. The arrows indicate whether the perfusion started at high or at low pressure.

Figs. 2–4. Renal blood flows (dotted lines) and urine flows (plain lines) in three experiments.

during the first stage, the curve of urine flow runs higher than during phase 2, whereas there is no similar difference to be seen in the blood flow.

In Exp. 22 (Fig. 3), two kidneys were perfused at the same ranges of pressure, kidney A starting from a low level, kidney B from a high level; the first kidney produces more urine, and its blood flows are higher. Exp. 23 (Fig. 4) was carried out under similar conditions: here, kidney A, with higher urine flows, has higher blood flows at the beginning and lower ones at the end.

Owing to the inconsistency of these results, we cannot conclude that blood flow may be more determinant than perfusion pressure. The question remains open.

The question arises whether the water is secreted through a purely physical force or through an active process. A detailed cataphoretic analysis of plasma proteins has been published in a previous paper (Brull & Nizet, 1953) and gave 39 g/l. of total proteins with 6·7 albumin and 93·3% globulin. This might mean a colloid osmotic pressure of about 6·5 mm of mercury or 85 mm of water. Since urine is secreted at pressures of 30 and 40 mm of water, this must be done against the osmotic pressure of the proteins, through an active process.

On the other hand, Bieter (1931) found that the kidney of *Opsanus tau*, also aglomerular, secretes urine at a pressure higher than that in the dorsal aorta. Yet this finding is irrelevant when *Lophius* is considered, since Nizet (in press) has shown that China ink injected into the arterial blood does not reach the tubules, going no farther than the capsulae. The blood supply to the tubules is conveyed solely by the renal portal veins.

Thus, admittedly, the tubules of *Lophius*, which have lost their glomeruli during the first stage of life, secrete water like a salivary gland. Have glomular kidneys lost such a property? This question will be worth discussing elsewhere, in the light of research done by one of us on the kidney of the dog.

Let us consider for a moment the *oxygen consumption* of the *Lophius* kidney. We already know (Brull *et al.*, 1953) that perfusion with oxygenated blood does not provide more, or more concentrated, urine than venous blood as it is collected from the fish. In the present experiments, our attempts to measure the degree of oxygenation of the blood have shown that the venous blood which normally irrigates the kidney contains no 'measurable' amount of oxygen. If an increased flow of arterialized blood acted merely by way of increasing the oxygen supply, it would be difficult to explain how a similar increase of venous blood supply would produce a result of the same magnitude.

It seems that the oxygen requirement of the *Lophius* kidney is very small. Its significance requires further study. Still, there are very active enzymic systems in such kidneys, considering they concentrate Mg about 50 times (with venous blood as well).

SUMMARY

The urine secretion of the kidneys of *Lophius piscatorius* perfused with heparinized *Lophius* blood is very sensitive to perfusion pressure below a critical level, above which it becomes insensitive. The response of the urine flow to pressure takes the form of an exponential curve.

The blood flow through the kidneys, while rising slowly at pressures of about 20–30 mm of water, responds arithmetically to pressure above such levels.

At present it is impossible to make out whether pressure or blood flow has the greatest influence on secretion.

Water secretion in the aglomerular kidney is an active process. The oxygen consumption of the *Lophius* kidney is unmeasurably low, yet remains a possible factor in secretion.

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J. Mar. biol. Ass. U.K. (1954) 33, 739–747
Printed in Great Britain

NOTES ON THE GROWTH AND BIOLOGY OF THE PRAWN *SPIRONTOCARIS LILLJEBORGII* (DANIELSSEN)

By R. B. Pike

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(Text-figs. 1–3)

The biology of the spirontocarids appears to have attracted little attention and the larvae of the European species are imperfectly known.

While working on the prawn *Pandalus bonnieri* Caullery (Pike, 1952), a considerable number of *Spirontocaris lilljeborgii* (Danielssen) were obtained on the same ground. This spirontocarid was infected by the bopyrid parasite, *Phryxus abdominalis* Kröyer. Collections of the prawn were made so that its biology, as well as that of the bopyrid, could be studied.

The author expresses indebtedness to colleagues at the Marine Station for assistance in preparing this paper, and to the Skipper and the crew of M.V. *Calanus* for their unfailing help in collecting material; to Miss E. R. Wallace for assisting in measuring specimens and to Mr J. N. Thomson for cutting and staining the sections.

NOMENCLATURE

Most European authors appear to have regarded *Spirontocaris lilljeborgii* as a distinct species from *S. spinus* (Sowerby). Appellöf (1906) does point out that although the two forms are usually distinct on the Scandinavian coast, intermediate forms have been obtained off Iceland. Kemp (1910) reviewed the situation while dealing with the Decapoda Natantia of Ireland and decided to call *S. lilljeborgii* a variety of *S. spinus* (*S. spinus* var. *Lilljeborgi* (Danielssen)). Holthuis (1947) has since reviewed the known species of Hippolytidae and has divided the genus *Spirontocaris* into further genera and given this variety specific status.

COLLECTION OF MATERIAL

All the prawns were collected from the Cumbrae Deep ground in the Firth of Clyde, Scotland. This ground is situated off the Isle of Bute (National Grid Reference 26/1159–1162), at a depth of 70–90 m. The gear used for collecting the adults was an 8 ft agassiz trawl having a net mesh of 1·3 cm bar; this was found to retain prawns of above 7 mm carapace length. A one-metre modified agassiz, working 15 cm above the bottom and fitted with a mesh of 1 cm bar was used for younger prawns, and the same frame fitted with a one-metre stramin net was used for the collection of larvae and early post-larval forms. It was difficult to obtain adequate samples of all size groups in a single day, especially in the summer when the finer mesh nets had to be used for the smaller size groups. Frequently, 2 days were required to obtain an adequate sample, and in each case the earlier date for sampling is given; the second sample was obtained within 7 days of the first sample.

MEASUREMENT OF MATERIAL

The standard measurement made was the 'length of the carapace', being the distance from the posterior margin of the eye socket to the dorsal posterior margin of the carapace. All early post-larval prawns were measured under the binocular microscope. The larger specimens were measured with fine draughtsman's calipers to the nearest 1 mm below and the data recorded in 1 mm groups.

TABLE I

Carapace length (mm)	Total length (mm)			No. of eggs
	Juveniles	Males	Females	
1	—	—	—	—
2	11·8	—	—	—
3	13·6	—	—	—
4	—	20·0	18·5	—
5	—	23·5	22·6	—
6	—	26·2	28·1	—
7	—	33·8	33·5	130
8	—	36·5	37·1	164
9	—	40·3	42·3	256
10	—	—	44·9	430
11	—	—	48·0	516
12	—	—	50·2	687
13	—	—	52·3	577

At the same time total lengths, tip of rostrum to the tip of telson were measured, and the arithmetic mean taken for thirty specimens in each mm size group. These means are shown in Table 1, together with the mean number of newly laid eggs taken from five individuals in each available mm size group.

GEOGRAPHICAL DISTRIBUTION

S. lilljeborgii is common in the Skagerrak and along the south and west coasts of Norway and has been recorded from Iceland (Stephensen, 1939, p. 17). It is not recorded by the same author (1928, p. 13) for the Faeroes, and he is doubtful of his East Greenland record (1912, p. 510). This doubt is also shared by Heegaard (1941, p. 42). Southward, *S. lilljeborgii* extends from the Irish Sea into the English Channel on the west and to the Norfolk coast on the east. *S. spinus*, however, is obtained from the Kattegat and along the whole west coast of Norway. It is recorded in small numbers from the Faeroes area, occurs on both the east and west coast of Iceland, and Greenland and as far north as Spitzbergen, Franz Joseph Land and Ellesmere Island. Its southern range is rather uncertain. According to Kemp (1910) only one rather atypical form was obtained from Irish waters. Dr N. S. Jones from the Marine Station, Port Erin, Isle of Man, informs me that he has obtained typical *S. spinus* from the Isle of Man, although *S. lilljeborgii* is more usually obtained. Thus in the British Isles the southern limit for *S. spinus* appears to be the Irish Sea on the west and possibly the Norfolk coast on the east.

OCCURRENCE IN THE CLYDE

As already stated, *S. lilljeborgii* occurs along with *Pandalus bonnieri* on a soft muddy substratum, inhabited by a community similar to that associated with *P. borealis* (Kröyer), as described by Hjort & Ruud (1938). I have already detailed (Pike, 1952) the main animal communities of this ground, and further details can be obtained from Allen (1953). Heegaard (1941) notes that *Spirontocaris spinus* in Greenland prefers shallow water (12–50 m) which is considerably shallower than its habitat in Spitzbergen. He suggests that this is possibly because *Balanus porcatus* da Costa, among which it hides, often occurs at Spitzbergen at greater depths than off the east coast of Greenland, and in both places *Spirontocaris spinus* is always found in association with this barnacle. As *S. lilljeborgii* does not occur in Greenland no similar observations were made for that species. *Balanus porcatus* is generally distributed within the Clyde, but does not occur in any number on the ground from which the specimens of *Spirontocaris lilljeborgii* have been obtained for this work, and no association with *Balanus* has been observed.

MIGRATION

The adults were extremely difficult to obtain during May and June, that is, just after the breeding season when they are moulting. It is thought that an inshore migration takes place at this time and that the prawns do not return again in numbers to the deeper water until July.

BREEDING

In the Clyde the ovaries of the female prawns begin to mature at the end of August (Fig. 1, A, 1) when the developing eggs can be seen through the cuticle of the thorax as sage green masses. Egg-laying begins late in November, and is about complete in a month's time. Incubation of the eggs lasts 3 months.

My records for 1951 show that on 21 November out of 100 females of *Spirontocaris lilljeborgii* captured, two were in berry. By 7 January 1952, nine out of 200 females carried eggs showing eye pigmentation, and on 19 February four out of 205 females had hatched their eggs. These dates and figures suggest

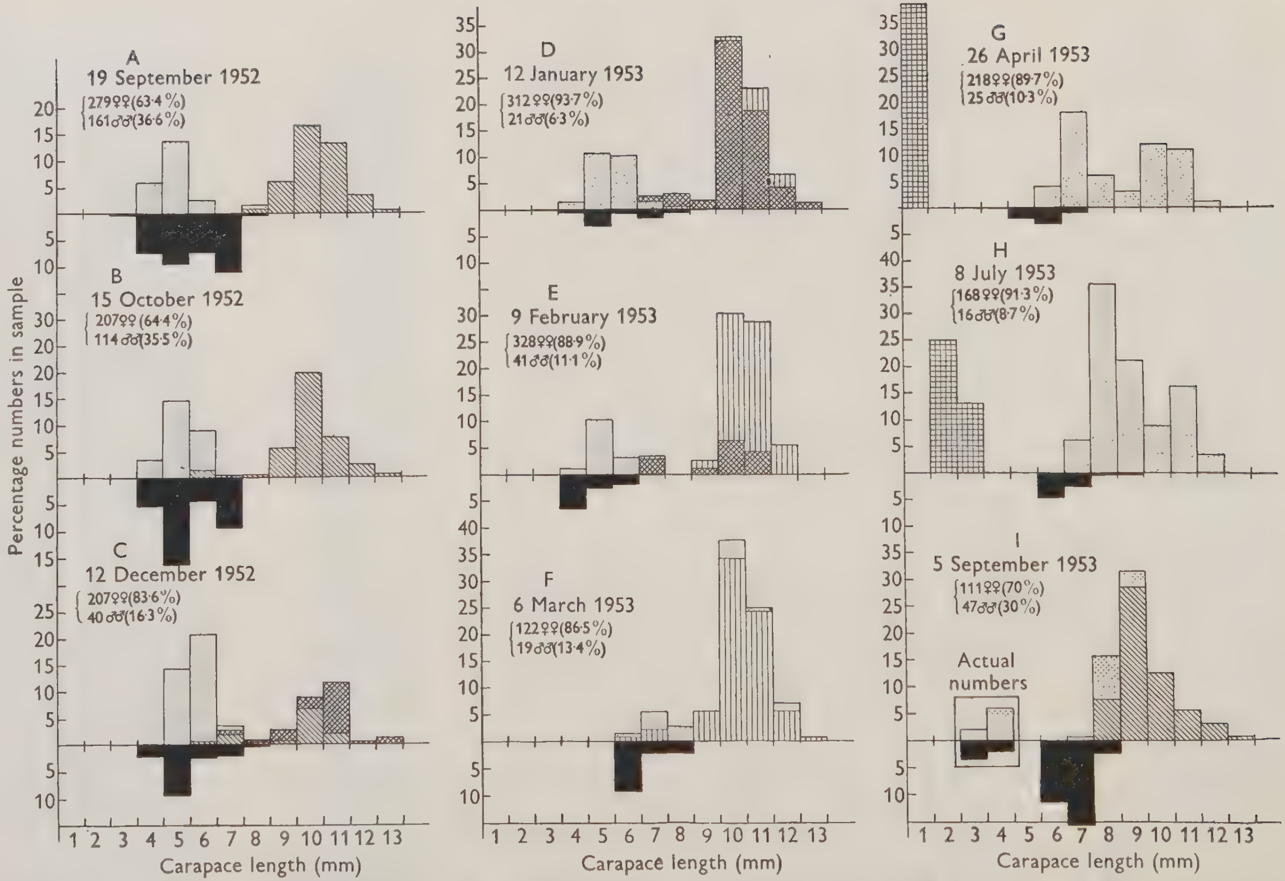

Fig. 1. Breeding in *Spirontocaris lilljeborgii*. ■, males (below the datum line); females: □, resting stage; ▨, ovaries green; ▩, carrying eggs; ▥, eggs with eye pigment; ▦, early post-larvae.

a total incubation period of 90 days. Egg-laying takes place about the time when the bottom sea temperature is highest (surface *c.* 9·6° C, bottom *c.* 11·5° C), and hatching when it is lowest (surface *c.* 7·1° C, bottom *c.* 6·5° C). Eye pigmentation first becomes visible in the egg about the 47th day after egg-laying. This is in keeping with the observations of Höglund (1942) who found that in *Leander squilla* (L.), kept in aquaria, eye pigmentation became visible half-way through egg development. The egg of *L. squilla* measures *c.* 0·45 × 0·59 mm whereas that of *Spirontocaris lilljeborgii* is much larger, *c.* 1·0 × 0·9 mm. There appears, however, to be little difference either in the time taken for the eggs to develop or in the number of larval stages in these two prawns.

Rate of Growth

The data obtained on growth are shown in graphic form in Figs. 1 and 2 and summarized in Table II. In preparing Fig. 2 the smallest and largest size has been shown for the age groups on the dates given in Fig. 1 and the lines that appeared to fit these drawn to show the general trend. The third year (II-group) is doubtful for both males and females, as the numbers are very small, and the difference in size might be explained by differential growths within the sexes during the second year. It did not seem advisable, on the data available, to work out the arithmetic means. The larvae of *S. lilljeborgii* were first recorded in 1953 on 5 March which is somewhat later than in 1952. In both cases hatching appeared to be complete in about a month. Records show that the larvae take 4–5 weeks to pass through the larval stages and become bottom living. They grow quickly and reach a carapace length of from 2–3 mm by early July (Fig. 1, H). Growth continues to be rapid until September when at a length of from 3 to 4 mm (Fig. 1, I) the males can be distinguished from the females by the shape of their pleopods. At this time the ratio of males to females is approximately 1 : 1.

By December the males have reached a carapace length of 4–6 cm and the females from 5 to 7 cm. From now onwards the females grow much faster than the males, as will be seen in Table 1 and Fig. 2. During the winter months growth almost ceases for both sexes.

The usual life span for males appears to be 18 months, with a possible maximum of 30 months. With the females 24 months is the usual life span, although a few appear to live for 36 months.

The majority of the first-year males are sexually mature by the first autumn and probably fertilize the females, while only the largest of this 0-group females are able to breed. (Fig. 1, B, C). By December of their first year the largest of the 0-group males have reached the same size as the smallest of the I-group males of the previous year. After the I-group male population has fertilized the females they appear to die (Fig. 1, C). After December it was difficult to obtain any males and it was not until September

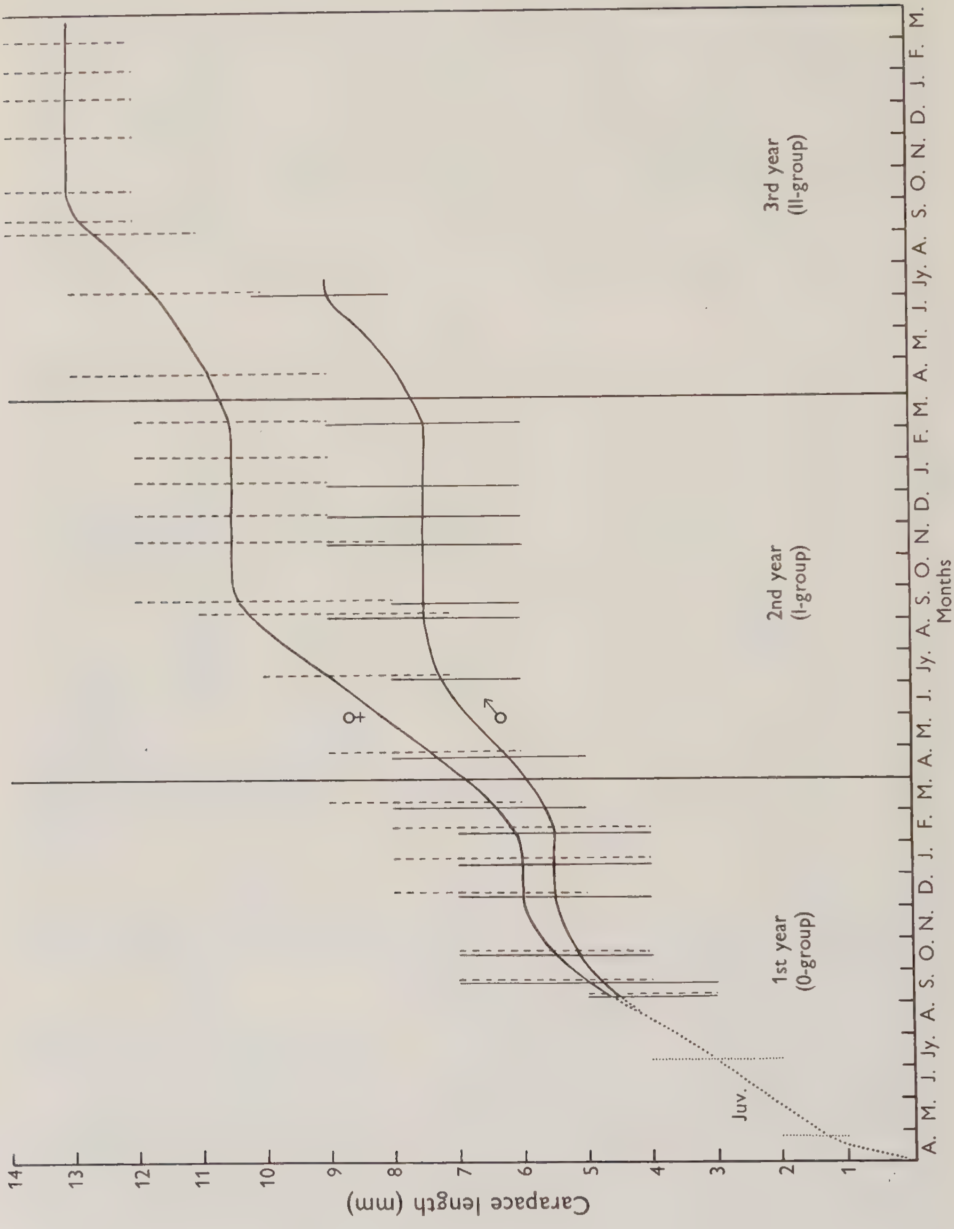

Fig. 2. Growth in *Spirontocaris lilljeborgii*.

following that a number of males were again collected (Fig. 1, I). This same scarcity of males has been noted over 2 years of sampling, and on no occasion has the collection of the second year (I-group) male population exceeded 30 % of the total female population. I have pointed out above that the ratio of males to females for the 0-group in September and October is roughly 1 : 1, and although later sampling may be inadequate the ratio is probably less than 1 : 2. It looks therefore as though some of the males die in their first year after fertilizing the females. To try and find out if this was the true ratio of males for the I-group for September 1953 (Fig. 1, I) sampling was carried out over 3 days with the 1-m agassiz using the 1 cm bar mesh, so that no small males should escape. By this means it was found that the ratio of males to females was just under 1 : 2. Such a method of collecting was too slow for general use, and the fine mesh was used for not more than half the collecting time.

TABLE II. GROWTH OF *SPIRONTOCARIS LILLJEBORGII*

	0-group		I-group		II-group	
Month of year	Age (months)	Carapace length (mm)	Age (months)	Carapace length (mm)	Age (months)	Carapace length (mm)
26 Apr. (G)	0–1	1·1–1·9	12–13	♂ 5·1–7·9 ♀ 6·1–8·9	24–25	♂ Absent ♀ 9·1–12·9
8 July (H)	1–4	2·1–3·9	13–16	♂ 6·1–7·9 ♀ 7·1–9·9	25–28	♂ 8·1–9·9 ♀ 10·1 12·9
5 Sept. (I)	4–5	3·1–4·9	16–18	♂ 6·1–8·9 ♀ 7·1–10·9	28–30	♂ Absent ♀ 11·1–13·9
19 Sept. (A)	4–5	♂ 3·1–6·9 ♀ 4·1–6·9	16–18	♂ 6·1–8·9 ♀ 8·1–11·9	10–18	♂ Absent ♀ 12·1–13·9
15 Oct. (B)	6–7	♂ 4·1–6·9 ♀ 4·1–6·9	18–19	♂ 6·1–7·9 ♀ 8·1–11·9	30–31	♂ Absent ♀ 12·1–13·9
12 Dec. (C)	7–9	♂ 4·1–6·9 ♀ 5·1–7·9	19–21	♂ 6·1–8·9 ♀ 9·1–11·9	31–33	♂ Absent ♀ 12·1–13·9
12 Jan. (D)	9–10	♂ 4·1–6·9 ♀ 4·1–7·9	21–22	♂ 6·1–8·9 ♀ 9·1–11·9	33–34	♂ Absent ♀ 12·1–13·9
9 Feb. (E)	10–11	♂ 4·1–6·9 ♀ 4·1–7·9	22–23	♂ Absent ♀ 9·1–11·9	34–35	♂ Absent ♀ 12·1–13·9
6 Mar. (F)	11–12	♂ 5·1–7·9 ♀ 6·1–8·9	23–24	♂ 7·1–8·9 ♀ 9·1–11·9	35–36	♂ Absent ♀ 12·1–13·9

SEX CHANGES

No change over from the male sex to the female sex has been observed. To check this point dissections of both sexes have been made and serial sections cut. The vas deferens of the male is a very thick and tightly coiled tube, and if any protandry occurred one would expect some trace of the vas deferens to remain after the change in sex had taken place, but this was not seen. The male pleopods are quite distinctive and intermediate forms should be easily seen during the examination of the male appendages. Lastly, serial sections through the male testis showed no trace of oocytes in the testicular tissue.

SECONDARY SEXUAL CHARACTERS

Höglund (1942) has given details of the breeding-dress in some Swedish eucyphideans (Caridea), and has described and figured the 1st pair of female pleopods for *S. lilljeborgii* during the breeding season, and during the rest of the year. I agree entirely with his findings, and for completeness have made drawings of these, from my material, together with the undifferentiated pleopods of the juvenile and also those of the adult male prawn (Fig. 3).

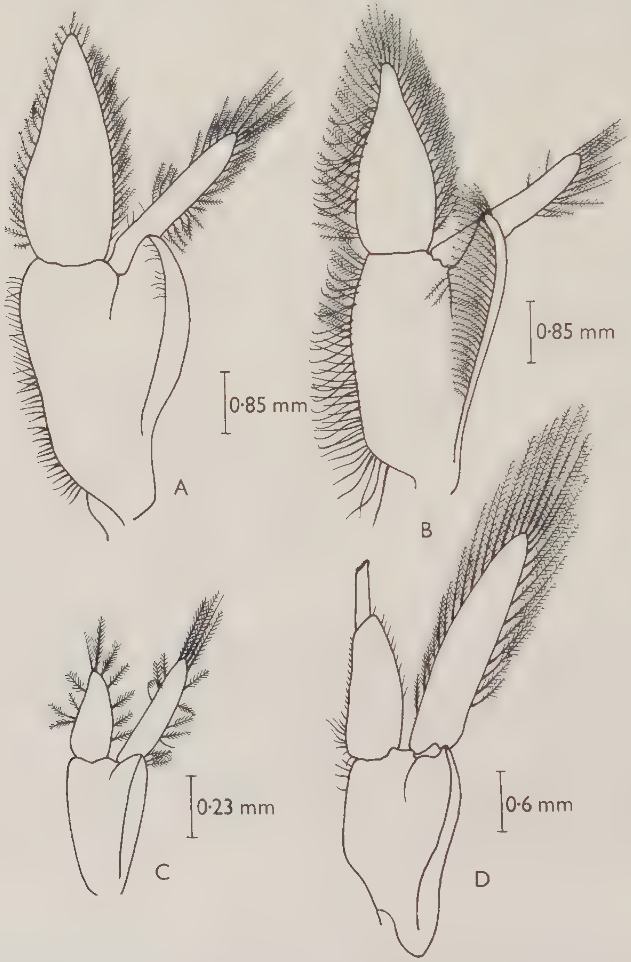

Fig. 3. The right pleopod of *Spirontocaris lilljeborgii*. A, female during the non-breeding season; B, female during the breeding season; C, juvenile; D, adult male.

SUMMARY

Spirontocaris lilljeborgii is a dioecious species and no protandrous hermaphrodites have been seen.

The majority of males become sexually mature in the first year (7–9 months). Some of these may die after fertilizing the females and the majority die after fertilizing the females for a second year (18 months old).

Only a small proportion of the females breed in their first year, all breed during their second year and a few appear to live for a third year.

A first-year female lays from 130 to 160 eggs, and the usual output of a second-year female is from 450 to 650 eggs.

Egg laying begins at the end of November and hatching is complete by the end of March.

The total incubation period is about 90 days. Eye pigmentation first appears at about half the incubation period (47 days).

Juveniles can be distinguished as males and females by the shape of their pleopods within 4–6 months of hatching.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ENZYMIC OXIDATION OF AMINES IN DECAPODS

By **H. Blaschko** and **J. M. Himms**

J. exp. Biol., Vol. 31, 1954, pp. 1–7

The occurrence of the enzyme amine oxidase in the tissues of *Eusepia officinalis* has already been previously reported. In the present work, a survey was made of the enzymic activity in the organs of two decapod cephalopods, *E. officinalis* and *Loligo forbesii*. In all organs of the alimentary tract, including the salivary glands, the liver and the pancreas, high enzymic activity was found. The enzyme was also present in nervous tissue, in the gonads, the renal appendages, the hearts, the pericardial glands and in other tissues. It was not found in muscle and skin.

The enzyme oxidized tyramine and related compounds, e.g. *p*-hydroxyphenylethanolamine (synthetic 'octopamine'), tryptamine and 5-hydroxytryptamine ('enteramine' or 'serotonin') and many aliphatic amines.

The liver of *Loligo* also contains the enzyme D-amino acid oxidase; this enzyme is already known to occur in *Octopus vulgaris* and in *Eusepia officinalis*.

H.B.

STUDIES ON *LYSMATA SETICAUDATA* RISSO (CRUSTACEA DECAPODA). III. ON THE ACTIVITY OF THE MOULT-ACCELERATING PRINCIPLE WHEN ADMINISTERED BY THE ORAL ROUTE. IV. ON THE SITE OF ORIGIN OF THE MOULT-ACCELERATING PRINCIPLE—EXPERIMENTAL EVIDENCE. V. THE OVARIAN INHIBITING HORMONE AND THE HORMONAL INHIBITION OF SEX-REVERSAL. VI. NOTES ON THE STRUCTURE OF THE NEUROSECRETORY SYSTEM OF THE EYE-STALK

By **D. B. Carlisle**

Pubbl. Staz. zool. Napoli, Vol. 24, 1953, pp. 279–85, 285–92, 355–72 and 435–47

The moult-accelerating principle of the eye-stalk of *Lysmata* is active when administered orally as well as upon injection; a far greater dose is required when the material is taken orally. It is present in extracts of both parts of the X organ, but was not found in extracts of sinus gland, of the retina and lamina ganglionaris or of the medullae interna and externa. Outside the eye-stalk it was found in extracts of the cerebral and thoracic ganglia. The hormone is probably a product of neurosecretory cells.

Eye-stalk ablation leads to an increase in ovarian growth and to an increase in the rate of reversal of sex from male to female in this protandrous hermaphrodite. Extracts of whole eye-stalks, of sinus glands or of the ganglionic part of X organs, when injected into intact animals intramuscularly, inhibit ovarian growth and depress the rate of sex reversal. Extracts of other tissues within the eye-stalk do not have this effect. The principle responsible for these effects is not active orally. Probably a single hormone is responsible for both effects—inhibition of ovarian growth and inhibition of sex reversal. This principle is presumably formed in the cells of the ganglionic part of the X organ and thence passed via the fibres of the sinus gland tract to the sinus gland where it is stored until finally released into the blood-stream. It is not the same as the moult-inhibiting or as the moult-accelerating hormone. The topography and anatomy of the neurosecretory structures which produce, transport and store these various hormones are described, and the relationship of their structure to the secretion of hormones discussed. A method is described by which it has been possible to observe the passage of neurosecretory material along the axons of the X organ connective; the entire axoplasm appears to be flowing along the axon. D.B.C.

ON THE RELATIONSHIP BETWEEN MAMMARY, SWEAT, AND SEBACEOUS GLANDS

By **D. B. Carlisle**

Quart. J. micr. Sci., Vol. 95, 1954, pp. 79–83

As a result of an investigation into the development of the mammae of male rabbits responding to regular injections of oestrogen and progesterone, it is suggested that the phylogenetic origin of mammary tissue lies in proliferated sebaceous glands. D.B.C.

SEISMIC PROSPECTING IN THE ENGLISH CHANNEL AND ITS GEOLOGICAL INTERPRETATION

By **M. N. Hill** and **W. B. R. King**

Quart. J. geol. Soc. Lond., Vol. 109, 1953, pp. 1–20

Recent geological information obtained by core sampling in the English Channel off the South Devon coast is set forth. This information has been combined with the results obtained from a seismic survey along a line southwards from Plymouth.

The evidence from these two sources points to the existence of a trough filled with New Red Sandstone deposits which extends from within a few miles of the English coastline to a point south of the middle of the Channel. The thickness of these deposits reaches approximately 3000 ft.

The line of reefs formed of metamorphic rocks extending from Bolt Tail to the west of the Eddystone projects through the New Red Sandstone formations, which appear to be swamping an irregular topography.

Southwards from the Eddystone the breccias and sandstones of the Permian and Trias are followed by Keuper Marl and a small thickness of Lower Jurassic formations. These in turn are covered unconformably by a few hundred feet of Chalk. M.N.H.

The Atrial Nervous System of Amphioxus (*BRANCHIOSTOMA*)

By W. Holmes

Quart. J. micr. Sci., Vol. 94, 1953, pp. 523–35

An interconnected system of neurons lies throughout the lining of the atrial cavity of *Branchiostoma*. Since the neurons lie in or immediately below the atrial epithelium, and are not confined to the gut wall, it is suggested that the homology with the craniate autonomic (enteric) system, proposed by Boeke, gives an incomplete interpretation of the system.

The atrial nervous system is connected with the central nervous system through the dorsal spinal roots, and contains receptor and effector elements. It is particularly dense in the walls of Lankester's atrio-coelomic 'funnels' and round the inner surface of the atriopore. The distribution of smooth muscle in the gut wall is discussed. Since the contents of the atrial cavity are a constantly refreshed sample of the external environment, and since the outer surface of the animal is so sparsely innervated, it is suggested that the atrial nervous system may play an important part in determining behaviour.
W.H.

Contraction and Relaxation in the Adductor Muscles of *PECTEN MAXIMUS*

By J. Lowy

J. Physiol., Vol. 124, 1954, pp. 100–5

The phenomena of contraction and relaxation in the adductor muscles of *Pecten maximus* have been studied by simultaneously recording muscle potentials and movement. The results confirm and extend the findings obtained in similar experiments with the adductor muscles of *Mytilus edulis*. Thus it was shown that in *Pecten* slow phasic contractions are accompanied by smooth muscle action-potentials whose number and amplitude depends on the extent of contraction, and that the maintenance of tension is associated with continuous electrical activity in the smooth muscle. The striated muscle works by large synchronous contractions. As in the case of the *Mytilus* adductor, a state of contraction in the smooth *Pecten* muscle can be terminated by nervous stimulation.

It is concluded that tonic contraction in lamellibranch smooth muscles is a tetanic phenomenon and that the slow speed of these muscles adequately accounts for their capacity to maintain a state of tension for long periods of time without appreciable fatigue. J.L.

FACILITATION IN SEA ANEMONES. III. QUICK RESPONSES TO SINGLE STIMULI IN *METRIDIUM SENILE*

By **D. M. Ross**

J. exp. Biol., Vol. 29, 1952, pp. 235–54

This paper describes further work on anemone extracts which in earlier experiments had the effect of enabling *Metridium* to respond to single stimuli. (In untreated animals a single stimulus elicits no response; it sets up facilitation and enables the animal to respond to subsequent stimuli.) The new information can be summarized as follows. (1) Extracts are most effective in causing responses to single stimuli from 15 to 30 sec after introduction, which is too soon for any material to reach the neuromuscular junctions where facilitation is set up. (2) There is no difference in the potency of extracts from stimulated and unstimulated animals, so the active substance in the extracts can have no relation to neuromuscular activity in the animals. (3) Other treatments have similar effects, big changes in pH, K ions, bile salts and saponin, but they have no obvious neuromuscular significance, suggesting that the effect is not due to any specific neuromuscular action.

The general conclusion is that these are not direct facilitating effects but arise from sensory excitation set up by the treatments. Yet there is a singular absence of spontaneous contractions and post-stimulus contractions, suggesting differences between this kind of excitation and the impulses set up by electrical stimulation. D.M.R.

RESEARCHES ON PHORONIDEA OF THE GULLMAR FIORD AREA (WEST COAST OF SWEDEN)

By **Lars Silén**

Ark. Zool., Ser. 2, Bd. 4, 1952, pp. 95–140

Four species of *Phoronis* were treated, viz. *P. pallida* (Schneider) (previously unknown in the adult form), *P. mülleri* Selys-Longchamps, *P. hippocrepia* Str. Wright, and *P. ovalis* Str. Wright. Material of *P. hippocrepia* collected during a stay at the Plymouth Laboratory in 1949 was used for comparison. The anatomy and histology of the different organs, except the nervous system, were investigated. The four species proved to represent as many distinct types. However, it was considered premature to erect separate genera for them as the phoronid species known are sufficiently few to be easily handled, and too few to allow of more than a very dim idea of the phylogenetic lines within the group. L.S.

ON THE NERVOUS SYSTEM OF *PHORONIS*

By Lars Silén

Ark. Zool., Ser. 2, Bd. 6, 1954, pp. 1–40.

Investigations were made on six species. *P. hippocrepia*, represented by material collected by the author at Plymouth in 1949, proved to be the form most suited for studying the histology in general of the nervous system. Fixation in Bouin's fluid and staining according to Bodian gave fairly selective results. Certain experiments were performed on living material. The nervous system is situated in the epidermis. Interior organs are innervated by fibres perforating the basal membrane. The chief constituent is a nerve net, typically developed in the metasoma, concentrated into a nervous ring (including the 'ganglion') at the border between pro- and metasoma. The components are described. There are two, one, or no decussating giant fibres ('lateral nerves') in different species. A paired lophophoral sense organ is described. The organization of the nervous system in *Phoronis* is easy to interpret from the functional, but difficult to interpret from the phylogenetic point of view. L.S.

BOOK REVIEWS

A Treasury of New Zealand Fishes

By **David H. Graham, F.R.M.S., F.Z.S.**

Published by A. H. and A. W. Reed, Wellington, N.Z. Pp. 1–404. Price 63*s*. 1953

In this book on New Zealand fishes the author claims to be catering for a very wide public including senior boys and girls at school, school teachers, university lecturers, sporting and commercial fishermen and fisheries naturalists, both amateur and professional. In so doing he provides a wealth of information, much of it anecdotal and not always as accurate in fact or precise in expression as it might be. The otter trawl is not a 'method of trawling'—it is a fishing implement. Sixty fathoms of wire are not needed to reach the bottom in 20 fathoms of water, though this may well be the best length of warp to use for effective fishing in such depth. It is really too much of a simplification to say that the swim bladder 'can be filled with air and gases by the fish relaxing the muscles surrounding it'. Such inaccuracies are numerous and detract greatly from the usefulness of the book.

Part I, consisting of nine introductory chapters, gives a brief résumé of fish anatomy and biology and of fishery economics. Part II extends to 127 chapters, each chapter, with few exceptions, being devoted to a single species of fish. No taxonomic descriptions are given, but each species is illustrated by either a photograph or a black and white drawing. Both photographs and drawings are of quite a high standard and will prove useful in making rough identifications; but the absence of detailed descriptions leaves the book unsuitable for arriving at precise determinations. In short, the reviewer agrees wholeheartedly with the publishers' statement that Mr Graham's work is pre-eminently a fireside book. As such, it is rather highly priced at three guineas.

G. A. Steven

The Sea Angler's Fishes

By **Michael Kennedy**

Published by Hutchinson & Co. (Publishers) Ltd., London. Pp. 1–524, with 13 colour plates and numerous black and white illustrations. Price 50*s*. 1954

This book has been written for the benefit of sea anglers, commercial fishermen and others concerned with the British sea-fish trade, all of whom will find it to be a mine of valuable information. Even professional fisheries biologists will discover much to interest them within its pages.

A great deal is known about many of our sea fishes but the information is mostly stored away in technical journals that are not readily available to the general public. Mr Kennedy has endeavoured to fill this gap by summarizing existing knowledge concerning the food, migrations, spawning habits, growth rates and other characteristics of the salt-water fishes of the greatest general interest. Detailed descriptions of the 120 or so species dealt with are also given, together with illustrations of most of them. To the subject-matter abstracted from technical and other printed journals the author has added considerable additional information acquired at first hand by himself or at second hand from those of his many friends who are well versed in the lore of the sea.

Short appendixes on sea angling and commercial fishing methods might well have been omitted, both subjects having been already adequately dealt with in numerous readily available publications. The reviewer would also have preferred the combined index-glossary to have been printed separately—but this, perhaps, is only a personal predilection.

No criticism of this well-written and well-produced book is implied in the further suggestion that a small handbook covering identifications of all British sea fishes—e.g. Jenkins's *Fishes of the British Isles*—would be a useful companion volume. Keen sea anglers are sure to encounter species other than those dealt with by Mr Kennedy. G. A. Steven

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1953–54

The Council have to record with very deep regret the death on 31 December 1953 of Dr G. P. Bidder, a Governor of the Association for nearly fifty years, and one of its greatest benefactors and most valued counsellors. An obituary notice will appear in the Journal of the Association.

During the year the Earl of Verulam has been elected a Vice-President.

The Council and Officers

Four meetings of the Council have been held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was nineteen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

The Council record with very great pleasure that Her Majesty the Queen has conferred the honour of knighthood on our President, Professor James Gray, C.B.E., M.C., F.R.S.

The Plymouth Laboratory

The museum on the top floor of the main building has been partitioned on the north side to form two research rooms, one of which will be used for sterilizing glassware for the culture of micro-organisms. The histological workroom has been improved by the addition of a new bench, microscope storage cupboard and electrical embedding oven. All this work has been done by direct labour.

The Council are pleased to report that H.M. Treasury have sanctioned a Capital Grant from the Development Fund to meet the cost of an extension to the library and the construction of a small building for the preparation and storage of specimens for the Sales Department.

The Aquarium

All the large tanks on the north side of the aquarium have been emptied and cleaned; some of the rockwork has been re-set and new rockwork provided where needed. The conger eels, dogfishes and nursehounds, which for many years had occupied the largest tank, have been transferred to the second largest, and their place has been taken by some of the most active fishes such as sea-bream, pollack, and large wrasses. This has greatly increased the attractiveness of our biggest tank, formerly occupied by so many normally

Photo D. P. Wilson

Research Vessel *Sarsia*

inactive species. There have been some other rearrangements in the aquarium designed to give most room to the more active fishes, with beneficial results to their health. The aquarium has continued to be very popular, especially with summer visitors.

Towards the end of the year the appearance of the Aquarium was greatly improved by the removal of the central tanks and screen, and the erection of a new screen above the tanks on the south side. As a result there is now a large dry hall giving excellent views of the tanks on either side and with an increased floor space for the visiting public.

Dr D. P. Wilson has published in the *Journal*, Vol. 32, no. 1, a further set of observations on the habits of some of the fishes. These include the first detailed account of the method by which the electric ray (*Torpedo*) captures and kills its prey, and an account of the previously unknown burrowing habits of the red band-fish (*Cepola rubescens*).

Research Ships

The *Sabella* was working regularly until the late summer, when she was off-surveyed at Plymouth on 10 and 11 September 1953 and returned to the Admiralty. The seven years' good service that she has given while on charter have made possible many valuable contributions to the science of the sea from the Plymouth laboratory.

During the year the *Sula* completed her Lloyds full special survey of hull and machinery. In order to keep the vessel fully classified considerable overhaul was necessary in the gear box and electrical system. Both the *Sula* and the *Gammarus* are in good condition and apart from time required for survey and routine maintenance have been in continuous and active service during the year.

The Council wish to record their thanks to Captain W. J. Creese and the crew of the *Sula* for carrying out so successfully the extra demands made upon them during the three months when a second vessel was not available.

R.V. *Sarsia*

The new research ship, built by Messrs Philip and Son Ltd., Dartmouth, and designed by Messrs Graham and Woolnough of Liverpool, has been named *Sarsia* and was launched by Mrs James Gray at the firm's Noss yard on Tuesday, 14 April 1953. Trials were carried out at the end of October and the vessel was taken over by the Association on 2 November 1953. Her principal dimensions are: length o.a. 128 ft.; length b.p. 115 ft.; main breadth to outside of plank 28 ft.; mean draft about 9 ft. 3 in.—giving a gross tonnage of 318·93 (95·02 tons registered).

The propulsive machinery consists of a main engine—National R4 AUM6 diesel—developing 330 b.h.p. at 486 r.p.m. with a propeller speed of 162 r.p.m. obtained through oil operated reverse-reduction gear. Maximum speed is just over 10 knots, giving an economic speed of about 9 knots at 130–140 r.p.m. (propeller). An auxiliary low-speed drive is also provided by a 20 h.p. variable speed electric motor geared to the propeller shaft through the main gear box. This motor provides the very slow speeds required for plankton work and can serve also as emergency propulsion at speeds up to about 4 knots in the event of adjustments to the main engine becoming necessary. The vessel's normal endurance range is 3000 miles.

There are two laboratories—a larger laboratory on the main deck forward under the wheelhouse and chartroom, and a smaller laboratory on the main deck abaft the engine-room casing. Both laboratories are provided with direct and alternating current at 220 V. Direct current supply varying from 6 to 72 V. can also be tapped from a battery of accumulators housed in a special accumulator compartment. Standard racks for carrying electronic equipment are fitted in both laboratories. In the forward laboratory there is a large gimbal table and a fitted dark room opens off the after laboratory.

Navigational aids with useful scientific applications consist of:

Decca Navigator type 5 with lane identification.
Decca Radar type 12 with 12 in. p.p.i.
Kelvin and Hughes Echo Sounder type M.S. 21*b* with range 0–720 ft. and 0–720 fm.
Kelvin and Hughes Echo Sounder type M.S. XXIe with a normal range of 0–2250 fm.
Marconi type 848 Recording Echometer with range of 0–140 fm.
Redifon Radio Telephone.

A three-drum trawl winch carries 500 fm. of $2\frac{1}{2}$ in. trawl warp on each of the two starboard drums and 1800 fm. of $1\frac{3}{8}$ in. wire on the port drum. A main hydrographical winch situated forward on the port-side under the forecastle deck carries 5000 m. of 4 mm. (diam.) wire on a single drum. A smaller two-drum winch on the after-end of the boat deck carries 500 m. of 4 mm. wire on one drum and 300 m. of 7 mm. wire on the other drum. All winches are hydraulically operated by Vickers-Armstrong's V.S.G. installations.

Two refrigerated cold chambers—one wet and one dry—are provided for scientific purposes, in addition to one domestic refrigerated store.

The ship's company totals sixteen, and there is cabin accommodation for four scientists in one double-berth and two single-berth cabins.

The Council wish to record their appreciation of the very valuable assistance given by Dr G. A. Steven during the building of this ship. They wish also to thank Captain C. A. Hoodless, D.S.C., and Chief Engineer, Mr R. S. Harrison, D.S.M., who were always in close consultation and latterly spent much of their time at Dartmouth, and Mr F. J. Warren who gave much help where electrical knowledge was required.

The Staff

Dr H. G. Vevers, M.B.E., has been promoted to the grade of Principal Scientific Officer.

Dr J. Lowy left the staff at the end of September on his appointment as Lecturer in Experimental Zoology at Queen's University, Belfast.

Dr J. A. C. Nicol has been awarded a Guggenheim Fellowship and he left Plymouth in September 1953 to work for several months at the Scripps Institution of Oceanography at La Jolla.

Mr N. A. Holme spent October and November 1953 in India, where he investigated a problem of underwater damage by boring molluscs at the request of a British firm of Civil Engineers.

Dr B. C. Abbott attended the International Physiological Congress in Montreal in September 1953.

Dr H. G. Vevers attended the meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1953.

Mr D. B. Carlisle attended the International Symposium on Neurosecretion held in Naples in May 1953.

The Council have pleasure in recording the award by Her Majesty the Queen of Coronation Medals to Mr F. S. Russell and Mr W. H. Searle.

Mr F. S. Russell, F.R.S., was elected President of the Devonshire Association for the Advancement of Science, Literature, and Arts for the year 1953–54 and delivered an address on the English Channel at the meeting held in Plymouth in June 1953.

Occupation of Tables

The following one hundred and forty workers have occupied tables at the Plymouth laboratory during the year:

E. Adams, Plymouth (Library).
W. H. L. Allsopp, British Guiana (General).
Señora A. Alvariño, Madrid (Plankton and fouling organisms).
D. C. Arnold, Oxford (Biology of Carapidae).
Miss D. E. Ashhurst, Oxford (General).
Dr Daphne Atkins, London (Ciliary feeding mechanisms).
Dr J. R. Baker, Oxford (Decapod crustaceans and Lucernarians).
Miss D. Ballantine, Development Commission (Taxonomy and culture of marine flagellates).
Dr R. H. Bhattacharyya, Calcutta (General).
Dr C. Bliding, Borås, Sweden (Marine algae).
B. Boczarow, Plymouth (Library).
A. D. Boney, Plymouth (Biology of *Pygmaea*).
E. R. Braithwaite, Plymouth (Library).
Prof. & Mme L. Brull, Liége (Physiology of kidney in *Lophius*).
Prof. R. Buchsbaum, Pittsburgh, Pa. (General).
W. Burke, London (Histological methods).
Dr R. W. Butcher, Burnham-on-Crouch (Culture of small algae).

Dr P. C. Caldwell, London (Giant nerve fibres in *Loligo*).
P. Childs, Plymouth (Comparative anatomy of biliary tract).
Dr P. N. J. Chipperfield, Brixham (Library).
B. Clarke, Leeds (Structure of flagella).
Miss E. Clay, Brixham (Library).
J. C. Cleverly, Cambridge (Geology of the English Channel).
R. D. Cohen, Plymouth (Library).
J. A. Cole, Cambridge (Geology of the English Channel).
D. J. Collins, Plymouth (Library).
C. A. Cosway, Torquay (Library).
C. B. Cowey, Oxford (*Nitzschia*).
R. I. Currie, National Institute of Oceanography (Oceanography).
Dr D. H. Cushing, Lowestoft (Development of pilchard eggs).
Dr Y. Cuypers, Liége (Physiology of kidney in *Lophius*).
Dr R. Phillips Dales, London (Biology of nereids).
Dr D. Davenport, Santa Barbara, Cal. (Physiology of commensalism).
A. A. Day, Cambridge (Geology of the English Channel).
Dr D. de Giusti, Wayne Univ., Detroit (Acanthocephalan parasites of *Gobius*).
Miss J. M. Dodwell, Birmingham (General).
Miss J. Donovan, Cambridge (Nerve net of sea anemones).
Mlle L. Dubois, Liége (Physiology of kidney in *Lophius*).
C. Edwards, Belfast (Ecology of *Marinogammarus*).
Prof. Regina Cubillos de Etcheverry, Valparaiso (Chemistry of sea water).
D. Etherington, London (Parasites of *Stolonica*).
M. A. Faithfull, London (Shore ecology).
Dr and Mrs J. Bruce Falls, Toronto (General ecology).
Major G. B. O. Farrington, Plymouth (Library).
Dr Maria Felinska, Buckden, Huntingdon (Ciliates).
Miss V. E. Ford, Brighton (Development of *Fucus* eggs).
Prof. Björn Föyn, Oslo (Colour pattern in *Pomatoceros*).
Cdr R. H. C. Frampton, R.N. (Rtd.), Plymouth (Library).
Dr Vera Fretter, London (Ion exchange in osmoregulation).
Mrs E. Gibbon, Yelverton (Library).
Dr D. R. Glasson, Plymouth (Library).
Prof. A. Graham, Reading (Prosobranch gastropods).
Surg.-Cdr P. H. K. Gray, R.N., Plymouth (Library).
Miss S. G. Greening, Birmingham (Physiology of *Arenicola*).
D. N. F. Hall, Colonial Fisheries (Plankton).
Miss M. F. Hall, Oxford (Behaviour of *Spinachia*).
Prof. B. Hanström, Lund (Neurosecretory cells in polychaetes).
Dr T. J. Hart, National Institute of Oceanography (Plankton).
B. T. Hepper, Conway (*Mytilicola intestinalis*).
Dr M. N. Hill, Cambridge (Geology of the English Channel).
Prof. A. L. Hodgkin, F.R.S., Cambridge (Giant nerve fibres in *Loligo*).
L'Abbé H. Hoestlandt, Lille (Colour variation in *Sphaeroma serratum*).
Dr W. R. Howells, Plymouth (Library).
O. D. Hunt, Newton Ferrers (Library).
R. F. Hutton, Miami Univ. (Fulbright Scholar) (Trematode parasites).
L. A. J. Jackman, Paignton (Library).
F. J. Jeffery, Plymouth (Library).
Dr C. H. Jellard, Plymouth (Bacteriological tests).

Dr A. Bohus Jensen, Copenhagen (Fertilization processes in *Ciona*).
D. Jones, Teddington (Opacity of sea water).
J. D. Jones, Bangor (Library).
L. W. G. Jones, Brixham (Library).
Dr G. Y. Kennedy, Sheffield (Porphyrins in marine animals).
Miss J. Kerslake, London (Systematics of *Ectocarpus*).
Dr B. H. Ketchum, Woods Hole (General).
Dr R. D. Keynes, Cambridge (Giant nerve fibres in *Loligo*).
M. C. Kingwell, South Brent (Library).
Sir Francis Knowles, Bart., Marlborough (Neurosecretion in crustaceans).
Dr F. Koczy, Gothenburg (General).
M. E. Korn, London (Holothurians).
Miss L. Lake, London (Ecology of buoys in Plymouth Sound).
D. H. Leaback, London (Cephalopods).
Dr M. V. Lebour, Plymouth (Decapod crustaceans).
J. Llewellyn, Birmingham (Fish parasites).
P. A. Longton, Plymouth (Library).
Prof. O. E. Lowenstein, Birmingham (Sense physiology in elasmobranchs and rockling).
Dr A. G. Lowndes, Plymouth (Bottom sediments).
Dr G. A. C. Lynch, Plymouth (Library).
G. O. Mackie, Oxford (Nervous system of coelenterates).
Prof. R. Magalef, Barcelona (General).
Prof. I. Manton, Leeds (Structure of flagella).
P. T. Marshall, Cambridge (General).
Mlle M. M. Martin, Paris (Marine animal painting).
Mrs J. B. Matthews, St Merryn (Library).
Prof. S. A. Matthews, Williamstown, Mass. (Endocrinology of fish).
Dr A. J. Matty, Nottingham (Endocrinology of elasmobranchs).
R. B. Mayne, Plymouth (Library).
D. A. McGill, Bucknell, Pa. (Fulbright Scholar) (Iodine metabolism in *Ciona*).
Dr J. E. Morton, London (Morphology of molluscs and ascidians).
Dr R. H. Murray, Birmingham (Sense physiology in elasmobranchs).
Dr Wheeler J. North, Cambridge and La Jolla (Light sensitivity of *Metridium*).
Mrs Maj Östbring, Lund (Chromatophores in polychaetes).
Miss G. Owen, Worthing (Coelenterates).
J. S. Owen, Sudan (Library).
Dr R. W. Owen, Leeds (Fish parasites).
Dr C. F. A. Pantin, F.R.S., Cambridge (Nerve net and light sensitivity of sea anemones).
A. H. Papworth, Northampton (Plankton).
Dr L. M. Passano, Washington University, Washington (Nerve net of sea anemones).
A. R. Paul, Eastbourne (Skeletal fibres in *Chalina*).
Dr H. H. Poole, Dublin (Photoelectric measurements of submarine illumination).
P. E. Potter, Bristol (Plant pigments).
Miss B. Rickard, Plymouth (Library).
Miss E. A. Robson, Cambridge (Nerve net of sea anemones).
Miss L. M. Rood, Teddington (Opacity of sea water).
D. P. Sharman, Birmingham (General).
R. J. Snell, Ruislip (Library).
Dr A. J. Southward, D.S.I.R. (Shore ecology).

Miss F. A. Stanbury, Plymouth (*Cladophora*).
Prof. J. H. Starling, Lexington, Virginia (General).
Ove Sundene, Oslo (Algae).
J. C. Swallow, Cambridge (Geology of the English Channel).
F. H. Talbot, Durham (Otoliths in *Raia*).
Dr J. T. Temple, London (Nervous system of *Hemimysis*).
H. Wilson Thomas, Plymstock (Library).
I. M. Thomas, Adelaide (Iodine uptake in lower chordates).
Dr G. Thorson, Copenhagen (*Scaphander*).
Prof. E. B. Verney, F.R.S., Cambridge (Physiology of kidney in *Lophius*).
I. Vigeland, Oslo (Bryozoans).
J. M. Warren, Uganda (General).
Dr G. P. Wells, London (Sedentary polychaetes).
Dr J. H. Welsh, Harvard (Acetylcholin bioassay; coelenterate toxin).
Miss M. R. Wheeler, Oxford (General).
Miss M. Whitear, London (Histology of fish skin).
J. H. Wickstead, Colonial Fisheries (Plankton).
M. S. Wills, Teddington (Opacity of sea water).
Mlle L. Wilsens, Liége (Physiology of kidney in *Lophius*).
H. V. Wyatt, Plymouth (*Calyptraea chinensis*).
Prof. J. Z. Young, F.R.S., London (Optic nerve in cephalopods).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Dr Hiroshi Tamiya, Tokio; Dr F. D. Ommanney, Singapore; Dr G. Rollefsen, Norway; Prof. Buzzati-Traverso, Pavia; Dr K. Pampapathi-Rao, Rajahmundry; Dr S. Jones, Calcutta; Prof. T. Katsuki, Japan; W. L. Necker, Chicago; Dr Fr. Hustedt, Germany; J. M. Thomson, Cronulla; Dr and Mrs G. Hoecker, Chile; Dr Björn Holmgren, Chile; Miss D. L. Ray, Seattle; Dr Dorothy Bliss, Harvard; Prof. H. R. Dew, Sydney; Miss L. E. Foster, San Francisco; P. B. N. Jackson, Northern Rhodesia; Prof. Tohru Uchida, Japan; Dr W. Schmitz, Germany; T. E. Allfree, Nairobi; Dr J. F. G. Wheeler, Zanzibar; Dr G. E. Maul, Madeira; Dr E. Steemann Nielsen, Copenhagen; Dr Warren Weaver and Dr J. George Harrar, Rockefeller Foundation, New York; K. Baalsrud, Oslo; Miss E. C. Pope, Sydney.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by thirty-nine students from the following Universities and University Colleges: Oxford, Cambridge, Edinburgh, London, Durham, Sheffield, Nottingham, Newcastle, Southampton, Aberystwyth, Cardiff, Exeter, and the Regent Street Polytechnic.

Also during the Easter Vacation Mr I. F. Thomas brought a party of four boys from Oundle School. In June Mr R. W. Webster brought fourteen students from Regent Street Polytechnic.

Dr G. E. Newell, Dr J. E. Forrest, and Dr J. E. Morton conducted a course in September for nineteen students from Queen Mary College.

A meeting of the British Phycological Society was held at the Plymouth laboratory during the last week of September at which the attendance was forty-two.

A special meeting was held in December at the Plymouth laboratory to discuss the present position regarding the possibilities of mass culture of marine unicellular algae. Dr F. N. Woodward, Director of the Institute of Seaweed Research, took the chair, and twenty-seven attended the meeting, including representatives of fourteen laboratories.

Scientific work of the Plymouth Laboratory Staff

Sea Water and Plankton

In conjunction with Dr H. H. Poole, who again visited the laboratory, Dr W. R. G. Atkins has worked on a method for measuring the extinction coefficient of light in the sea using two submerged photometers and thus avoiding certain errors inherent in a comparison with a cell in air.

They have done further work on the scattering of light in sea water, studying in particular the effect of the colour of the light and of filtering the water with collodion membranes of different average pore diameters; a triple filtration ending up with a filter 0·1 μ A.P.D. gave water with slightly less scattering than a fairly good sample of distilled water. Differences can be detected from sample to sample.

Dr Atkins, Mr F. J. Warren and Miss P. G. Jenkins have studied the depth of visibility of a 20 cm. Secchi disk in relation to the quantity of chlorophyll in the upper 20 m. of sea water at station E1 for two years. The relation is an inverse one; the results are often surprisingly concordant, but the admixture with deeper water, when the autumnal cooling leads to an isothermal column, introduces a discrepancy as one would expect.

The very clear water found in June was identical in salinity with the less clear May water, but coincided with the summer minimum of chlorophyll. The phytoplankton scatters light and reduces visual range, while offering little obstruction when diffuse light is measured as it falls on a horizontal plate.

Miss Jenkins has continued the work on the phytoplankton at station E1 by means of spectrophotometric determinations of chlorophyll and the identification of the organisms by obtaining cultures from nine depths every month, or fortnightly at important times. This essential link between the living organisms and the effects due to them has heretofore been lacking at station E1. Especially with the nanoplankton the changes may be very rapid and cells may accumulate near the bottom.

Mr F. A. J. Armstrong has continued the regular cruises to station E1 and analyses of water samples for phosphate, total phosphorus and silicate. These cruises have been more frequent in 1953 than earlier years, and samples have been taken at extra depths. The results of the analyses for the four years 1950 to 1953 have been cast into a graphical form for publication in the *Journal*. A set of samples taken for Dr Cooper by U.S.S. *Rehoboth* has also been analysed for phosphate and silicate. Mr Armstrong has assisted Dr Wilson with the chemical and bacteriological part of his experiments on biological differences between sea waters.

Dr H. W. Harvey has examined a wide range of experimental data bearing upon the factors which affect the growth rate of phytoplankton, which embraced a voluminous literature dealing with algal metabolism. It was hoped to find possible reasons for many apparently anomalous observations which might help in designing future experiments.

Dr Harvey has rewritten much of his book, *Recent Advances in the Chemistry and Biology of Sea Water*, with the aim of presenting the inter-relations between composition of the water, plant production and the animal community, which are becoming clearer through investigations made in several countries since the first edition was published seven years ago. These embrace work done in the Plymouth laboratory on the quantities and forms in which phosphorus compounds occur in the water, plants and animals, their change with the seasons and from place to place—an inquiry which is now also being pursued in the tropics and eastern Atlantic and by the Ice Patrol in the Arctic in order to differentiate water masses. A reason for correlating the many and diffuse observations, necessary for rewriting a general account of changes taking place in the sea, is to foresee better what future lines of research are likely to prove most productive. Some preliminary work has been done on methods of estimating zooplankton populations below unit area of the sea in terms of weight of organic matter or value as food for other animals; it is hoped to continue this in collaboration with other members of the staff.

In reports since the war the search for an understanding of the relative richness in the 1920's of the English Channel in plant nutrients and associated rich production of animals has been described. Dr L. H. N. Cooper has now been able to erect a generalized hypothesis relating conditions in the English Channel to the degree of coldness of Arctic winters—the hypothesis of upward displacement.

In part, Atlantic deep water is formed by cooling of saline water derived from the North Atlantic Drift system. Only around southern Greenland and probably in the Norwegian Sea can this water be formed in bulk. In the first two decades of this century a succession of cold Arctic winters probably led to considerable formation of deep water which could be accommodated in other parts of the Atlantic only by upward displacement of the water already there. This should have led to nutrient enrichment of the upper waters by physical processes which may operate always. The Arctic winters of 1918, 1920 and 1921 were exceptionally cold and should have given rise to exceptional upward displacement. Consequently, surface enrichment with nutrients should have reached its maximum in 1921. Since then there has been a long succession of mild winters, presumably reducing (*a*) the amount of deep water formed, (*b*) the extent of upward displacement, and (*c*) surface enrichment with nutrients of the eastern North Atlantic.

The impoverishment of the English Channel which set in after 1930 may have been initiated by exceptional cascading in that year due to a local cold winter and strong east winds. There is, however, an inconvenient phase difference of one year. All the work on the subject since 1946 has now fallen into place.

During 1953 the generous co-operation of U.S. Navy Hydrographic Office enabled U.S.S. *Rehoboth* to obtain essential information from fifteen deep stations in the Bay of Biscay and more is to come from deep stations worked west of Ireland. From these it is hoped to establish the southward course of Arctic water during mild winters. A number of corollaries which follow from the hypothesis of upward displacement are being investigated.

In 1945 Dr Cooper co-operated with Major Grange Moore of the Army Photographic Research Unit who was then evolving his brightness profile method of assessing beach gradients by aerial photography. Extinction coefficients of sea water in tubes determined by the Pulfrich photometer were notably greater than by the brightness profile method. Later it was found that the Pulfrich method also gave much higher values than those based on underwater measurements of vertical illumination at sea. An explanation can now be based on the work of Poole and Atkins on forward scattering of light and on the geometry of the light paths in the several methods.

Dr D. P. Wilson's and Mr F. A. J. Armstrong's experiments on biological differences between natural sea waters were continued during the 1953 breeding season of *Echinus esculentus*. Again Clyde water was compared with water from the English Channel at the international hydrographical station E 1. This year the two water masses were closely similar in their effect on the structure and health of the *Echinus* larvae reared in them, but the larvae were slightly better formed and a little healthier in the Clyde than in the E 1 water. Various experiments were made in an attempt to narrow the field within which must be sought an explanation of the marked differences previously observed. The 1952 experiment with active carbon was repeated, for it had been found that the carbon then used had contained a significant amount of copper. A copper-free carbon was obtained for comparison, with some differences in the results. A partially successful attempt was made to filter the water free from bacteria at the time of collection. An account of these and other tests is being prepared for early publication in the *Journal*.

During 1953, Mr P. G. Corbin has observed no change from the prevailing low level of macroplankton production off Plymouth as sampled by half-hour hauls with the 2 m. stramin ring trawl.

The monograph on the Medusae of the British Isles by Mr F. S. Russell has now been published by the Cambridge University Press.

The collection of species-pure cultures of marine phytoplankton organisms has been maintained by Dr Mary Parke throughout the year and a number of new forms have been isolated. Work is continuing on new members of the Chrysophyceae with three flagella.

During the year cultures for research purposes have been sent abroad and also to institutions in this country. A great number have also been produced for the use of research workers in the laboratory.

Miss D. Ballantine, on a Development Commission Research Grant, has been carrying out experiments in a constant temperature room on the growth of flagellates, estimated by increase in numbers, under various light intensities using fluorescent lamps. Growth curves for one organism are strictly comparable from one set of experiments to the next, but results obtained using different organisms vary widely.

Miss Ballantine has also continued her work on the descriptions and life histories of two undescribed species of *Gymnodinium* from the culture collection.

Macro-Fauna and Flora

The results of Dr D. P. Wilson's experiments in 1952 on the settlement of *Ophelia bicornis* larvae have been published in Vol. 32, No. 1, of the *Journal*. It was in 1952 that it was first clearly demonstrated that an attractive factor can be transferred from fresh Bullhill Bank sand to a neutral acid-cleaned sand. During the summer of 1953 many experiments were made with the object of obtaining further information about the nature of this attractive factor. It is expected that when analysis of the results is completed it will become clear that living micro-organisms play an indispensable role in making a sand attractive to the larvae of *O. bicornis*.

Mr G. M. Spooner has continued systematic and faunistic work on isopods and amphipods. An additional species of *Sphaeroma*, newly recognized on the Continent, proves to be prevalent in Britain. It has to be split off from the *S. serratum* of British authors. It has a preference for lowered salinity, with an optimum between that of *S. serratum* (marine) and *S. rugicauda* (mid-estuarine). To it belong the '*serratum*' of the Tamar Estuary.

The amphipods are being examined with a view to a revised list for the Plymouth Area, a considerable reference collection having been built up, from which the existing museum collection can be supplemented. Some quantitative counts are being made of amphipod populations of sessile epiphyte growths on rocks hand-collected by Mr G. R. Forster beyond low-water level.

Assistance has been given in determining critical species in collections from east Scotland and the Channel Islands.

Further collections of *Jassa* have been examined, including some from east Scotland and South Africa. The identity of the form in this country recently recognized as *J. valida* Dana with the native South African species has been confirmed. Material is now being assembled for writing up.

Additional information has been obtained by Mr Spooner on the occurrence of commensalism in the polynoid genus *Harmothoë*, the existing published records for the Plymouth area being unexpectedly incomplete. The most plentiful species is *H. spinifera*, which can be found not only in shale crevices intertidally, associated with *Amphitrite gracilis* (as found recently by Dr D. Davenport), but occurring freely in Plymouth Sound as a commensal of *Polycirrus caliendrum* inhabiting cavities in loose stone and clinkers. Normally the dredged material is not split open and this association has remained unnoticed hitherto.

The better-known *Harmothoë lunulata*, found in the tubes of various burrowing invertebrates, has so far produced three populations of more or less distinct colour pattern: (*a*) with *Amphitrite johnstoni* as host, in the Yealm Estuary; (*b*) smaller specimens with host *Acrocnida* and larger with *Leptosynapta*, at Salcombe; (*c*) with *Arenicola marina*, at Salcombe. The last is easily recognized by its dark colour and is var. *arenicolae* St Joseph. Small individuals of this species can be obtained from Plymouth Sound, but it is not clear whether these are commonly free-living: one has been found associating with *Thalassema neptuni*.

Dr H. G. Vevers has continued his studies on the biology of the echinoderms, and in collaboration with Dr G. Y. Kennedy, of the University of Sheffield, has published a paper in Vol. 32, No. 1, of the *Journal*, on the isolation and estimation of protoporphyrin in the integument of *Asterias rubens*. The relative amounts of this pigment in different colour types of *A. rubens* were determined by a fluorimetric method, and were found to decrease rapidly in the series: violet-brown, dark-brown, red and pale. Neither a porphyrin nor a porphyrinogen was found in the soft parts of the queen scallop (*Chlamys opercularis*) or mussels (*Mytilus edulis*) which form the main food of *Asterias rubens* in the Plymouth area. The source of the integumentary porphyrin is still uncertain, but it is suggested that it may be derived from the chlorophyll pigments found in the digestive caeca. Further work is now being done on the occurrence of porphyrins in other marine invertebrates.

Dr Vevers is also continuing his work on the carotenoid pigments of marine invertebrates, with a view to tracing their origin and function, and has started an investigation of some algal pigments. He is also designing new improvements to the lighting system of his underwater photographic apparatus, and it should be possible to test these at sea during the coming year.

Mr N. A. Holme has completed a paper on the biomass of the bottom fauna at Plymouth, which has been published in Vol. 32, No. 1, of the *Journal*. The mean dry weight of living matter at twenty stations was 11·2 g./m.^{2}. Much of this weight (64·4%) was made up of relatively large individuals weighing more than 0·2 g. dry. Polychaetes, lamellibranchs and echinoderms together made up about 80% of the total dry weight.

An attempt was made to compare the 1950 populations with those found by E. Ford in 1922–23. There seems to have been an appreciable overall decline in numbers of lamellibranchs, the group studied in most detail, since the previous survey. The lamellibranchs *Abra alba* and *Cultellus pellucidus* showed a considerable drop in numbers, as did the echinoderm *Echinocardium cordatum*. The decline in numbers may be due to the change in the hydrographic conditions in the Plymouth area which occurred in the early 1930's.

Work has now been completed on the ecology of the three species of *Ensis* found in British waters, a report of which has been published in Vol. 33, No. 1, of the *Journal*. Three ecological factors seem to be mainly responsible for the distribution of each species: wave-action, soil-grade, and depth. Tolerance of wave-action is dependent on the stability of a sandy beach; *Ensis siliqua* is much more tolerant of wave-exposure than either *E. ensis* or *E. arcuatus*. Where wave-action on a beach is too great, a species may be restricted to shallow water offshore. All three species have distinct preferences for soil of a particular grade. *E. arcuatus* occurs in soils typically containing nearly 30% over 1 mm. particle size, while the other species occur in soils where there is only 2 or 3% over this size. All species occur in a fairly wide range of grades, so that *E. arcuatus* may sometimes be found in the same place as either or both the other species. The three species occur on the shore and in shallow water offshore, *E. siliqua*, alone, having a definite lower depth limit of *c.* 20 m.

Some differences in ecology were noted on the south side of the English Channel compared with south-west England. It is possible that separate populations with slightly different habitat preferences occur.

Mr D. B. Carlisle has continued his studies on the Plymouth and Naples tunicates and contributed papers on this subject to the *Journal*, to the *Pubbl. Staz. Zool. Napoli*, and to the *Proc. Zool. Soc. London*.

Mr G. R. Forster has completed two short papers on the *anchor* dredge and on peritrophic membranes in the *Caridea*; these have been published in Vol. 32, No. 2, of the *Journal*.

During the summer he continued his investigation of sublittoral rocky shores by diving, whenever possible. Six dives have been made off Stoke Point, in which the sub-*Laminaria* zone has been reached, at a depth of approximately 10 fathoms below L.W.S.T. The occurrence and distribution of the more conspicuous forms have been observed down to a maximum depth of 14 fathoms.

The sublittoral gulley in Wembury Bay surveyed by Kitching, Macan & Gilson (*J. Mar. biol. Ass. U.K.*, Vol. 19, No. 2) in diving helmets in 1931 has been re-examined by Mr Forster. The most successful collecting method has been to split off slabs of the slate rock with a lead hammer and cold-chisel. Many small Crustacea have been taken in this way, as they remain clinging to the sponges and other sessile forms.

Early in the year, Mr P. G. Corbin obtained several artificial fertilizations of the eggs of the Atlantic smooth sand-eel, *Gymnammodytes semisquamatus*, from which larvae were successfully hatched. Earlier work by Einarsson had shown that one of the post-larval sand-eels commonly occurring in this area belonged to the above species, although formerly it had long been attributed to the greater sand-eel, *Ammodytes lanceolatus*. The larvae from the artificial fertilization provided independent confirmation of Einarsson's identification. There remains the interesting anomaly that in this area the young stages of the commonest species, the lesser sand-eel, *A. tobianus*, have not yet been seen. The young stages previously attributed to *A. tobianus* were shown by Einarsson to belong to *A. lanceolatus*.

Mr Corbin has been re-examining Saville-Kent's long overlooked notice of a fourth species of sucker fish, *Lepadogaster couchii*, in the British fauna. His account was preliminary and incomplete, but it leaves no doubt that he referred to a species which occurs in much the same region of the shore as the commoner Cornish sucker, *L. gouani*, but in the rather different habitat afforded by the cover of bushy weeds, particularly the *Cystoseira* spp., in preference to the under side of stones where *Lepadogaster gouani* is commonly found breeding early in the year. Although the species resembles the off-shore *L. bimaculatus* with which it was earlier confused, it has several readily distinguishing characters in addition to an entirely different habit and habitat.

Mr Corbin has continued his observations on the Lucernariidae, in particular by fortnightly or monthly searches at Wembury and Looe, which are providing data on seasonal abundance of the species.

No further work on the large mid-water net was undertaken this year by Mr Corbin and Dr Steven after the trials made in the summer of 1952 aboard R.R.S. *Discovery II*. It was considered that the work could not profitably be advanced until the facilities of the new ship were available.

For forecasting purposes Dr G. A. Steven has continued his regular sampling of landings from the Newlyn (Cornwall) spring mackerel fishery. Returns for the 1953 season were in encouraging agreement with his prediction of a moderately successful fishery. Unfortunately, the prospects for the 1954 season are rather less favourable than those for the previous two years.

Dr Steven is also collaborating with Mr L. A. J. Jackman of Paignton, Devon, in preparing a report on the part that appears to be played by temperature in regulating the arrival and departure of mackerel in inshore localities in Torbay. Dates of first arrival have been noted over the 9-year period 1945–53 and departure dates from 1941 to 1952 inclusive. The records reveal strikingly close correlations with sea surface temperatures. With only one exception mackerel made their first appearance when the sea temperature was within the range 11·1–11·6° C. In six different years the temperature was exactly 11·6° C. Over the 12-year period of departure records (with the exception of 1945) mackerel left the area when the temperatures were between 12·2 and 13·9° C. with a mean value of 12·9° C., the departure dates ranging from 24 September in 1952 to 11 November in 1949.

The trawling survey of the grounds in the vicinity of Plymouth, mentioned in last year's report, is nearing completion. Present catches continue to be better, on the whole, than those of the 1913–14 period and poorer than those of the 1920's.

Careful records of young fish catches in standard hauls with the 2 m. stramin ring trawl at a fixed position have been made from 1924 onwards. No comparable records exist for trawled catches of the fish after they have become large enough to be captured by this method. Dr Steven has, therefore, begun to record total catches of demersal fish from standard trawl hauls at international station L4 off Plymouth as nearly as possible at weekly intervals. After some years the data so collected should be of great interest and value.

Experiments are also being carried out by Dr Steven with the preparation of an artificial bait for crab-pots. The bait is easily made, easily stored, and retains its properties for a year or more. If it proves sufficiently attractive to crabs, lobsters and prawns it should be useful to fishermen at times when natural bait is scarce. A sample of the artificial bait kept for four years was still wholesome but had lost its power to attract prawns.

The compilation of a preliminary Check-List of the British Marine Algae has been completed by Dr Mary Parke, and the list was published in Vol. 32, No. 2, of the *Journal*. The separate collections of marine algae in the laboratory's museum have now been incorporated into a single catalogued collection. This has involved considerable checking of old specimens and remounting for better preservation. Miss D. Ballantine has given much assistance in this work, which is now more than half finished.

To fill gaps in the collection many specimens from the Devon and Cornwall coasts have been added, and it is hoped that eventually the Herbarium will be representative of all British coasts.

A close watch is being kept for new occurrences or increasing numbers of more southern forms along our coasts, and some new records have in fact already been made.

Physiology of Marine Organisms

The majority of marine animals, perhaps all, have some means of controlling the emission of light. Among recognized mechanisms are: nervous control of secretion; regulation of intracellular oxidation; muscular contraction of reservoirs containing luminescent material; and muscular control of shutters, screens, etc., for exposing or covering light organs. Detailed information has now been obtained by Dr J. A. C. Nicol for two such mechanisms in polychaetes, viz. nervous control of secretion (expulsion of luminescent material) in *Chaetopterus*, and regulation of intracellular oxidation in Polynoinae. The course of fatigue of the luminescent response in *Chaetopterus* presents certain interesting features, since the intensity of response which can be evoked by successive stimuli progressively declines to a low level long before the exhaustion of luminescent material. There is good reason for believing that the expulsion of luminescent material is effected by protoplasmic contraction of the cell periphery. Quantitative recording has permitted an analysis of the factors involved in fatigue, which resolve themselves into: reduction of luminescent material present; fatigue of contractile mechanism under rapid stimulation; decreased efficiency of the contractile mechanism, with diminution in volume of intracellular contents. This last factor is mainly responsible for the decline in intensity of successive, discrete responses.

Further studies have now clarified many features of the luminescent responses of polynoids. The normal luminescent response of an elytrum is a series of flashes which can be evoked by a single electrical shock. This rhythmic flashing is controlled by a peripheral ganglion in the elytrum, from which nerves radiate to the light cells; preparations lacking this ganglion give only a single flash to each stimulus. Consecutive flashes, following each other at suitable intervals, show an increase in intensity. This is in part due to summation when the frequency is high enough to permit fusion, but is to a great extent attributable to facilitation. This process, lasting as long as 4 min., occurs peripherally at the neuroglandular junction or in the photocytes. The time relations of the flashes have been determined from oscillographic records, and the course of fatigue in the rhythmicity of the underlying neural mechanism investigated. This experimental work, together with further observations on the effects of various salt solutions and biochemical agents on luminescence, has been published in Vol. 33, No. 1, of the *Journal*.

Dr Nicol has also written up his experiments, carried out in collaboration with Dr D. Whitteridge at Oxford, on conduction in the giant axon of *Myxicola*. This work deals with the course and speed of conduction in the giant nerve fibre of *Myxicola*, and the relation of conduction velocity to axon diameter. An abstract of the work appeared in Vol. 29, No. 1, of the *Journal*, and it is being published in full in *Physiologia Comparata et Oecologia*.

Dr J. S. Alexandrowicz has continued his studies of the muscle receptors in crustaceans. In *Squilla mantis* the presence of these organs in twelve segments has been established. The two nerve cells and the muscle fibres in each half segment differ in their structure, and in this and many other respects the receptors in stomatopods resemble those in decapods, having however certain peculiar features of their own. The results are being published in *Pubblicazioni della Stazione Zoologica Napoli*.

In *Leander serratus* muscle receptors have been found in five abdominal segments and in the thorax. The latter show an unusual arrangement in that four nerve cells are connected with one thin muscle bundle. The receptor elements of another category, called N-cells, have also been observed in *Leander* and found to be comparatively very large in this species.

The occurrence of muscle receptor organs has also been established in amphipods (*Marinogammarus marinus*), isopods (*Ligia oceanica* and *Idotea emarginata*), and mysids (*Praunus flexuosus*). In all these species the receptors are situated at the level of the extensor muscles on their dorsal surface, or near to it, and appear to be of essentially similar structure to those of decapods and stomatopods.

Dr Alexandrowicz has also investigated the innervation of the heart in the amphipod *Marinogammarus marinus*. Several systems of nerve elements have been found supplying the heart, viz. a ganglionic trunk situated on the dorsal heart wall, two pairs of nerves coming from the central nervous system and nerves for the arterial valves. There are, in addition, special nerves supplying the pericardial muscles and two trunks running in the pericardial wall, presumably belonging to the category of the pericardial organs.

Dr J. S. Alexandrowicz and Mr D. B. Carlisle have published in Vol. 32, No. 1, of the *Journal* an account of the experiments showing the effects of the extracts of the pericardial organs of decapods and stomatopods on the heart rhythm of these animals. Further experiments are being made in order to determine the nature of the active principle in these organs. The evidence at present suggests that this is not adrenalin, noradrenalin, tyramine, acetylcholine (though this is probably present in extracts), nor 5-hydroxytryptamine, but in some of its properties it is very like this last.

Mr D. B. Carlisle has continued his investigations into the endocrinology of moulting and sex in prawns and crabs. The moult-accelerating hormone is produced by the nerve-cells of the ganglionic portion of the *X* organ of the eyestalk and passed within the axons to the distal part of the *X* organ where it is stored until released. Reversal of sex and ovarian development in the Mediterranean prawn *Lysmata* have been shown to be under hormonal control; probably the same hormone inhibits both (*Pubbl. Staz. Zool. Napoli*, Vol. 24). No evidence has been found for the existence of the eyestalk moult-inhibiting hormone in *Leander*, nor in *Carcinus* in the moulting season, in which species it is known to exist outside this season. The structure and relationships of the endocrine structures of the decapod eyestalk have been investigated comparatively in collaboration with Dr L. M. Passano and a brief account published in *Nature*. A preliminary account of these organs in *Lysmata* appeared in *C.R. Acad. Sci. Paris*, and a fuller account has now been published in *Pubbl. Staz. Zool. Napoli*. A letter to *Nature* by Mr Carlisle and Sir Francis Knowles has proposed a simplification in the nomenclature of certain nervous and endocrine structures of crustaceans—the 'neurohaemal' organs.

Dr B. C. Abbott has studied the concentration by marine organisms of certain radioactive isotopes resulting from uranium fission processes. Attention has been mainly directed to the uptake of yttrium by cultures of *Nitzschia closterium* forma *minutissima* under various conditions of growth, illumination and aeration. The exchange of caesium and potassium between the external medium and living cells has also been studied in these unicellular algae and also in invertebrate muscle tissue.

Iodine accumulation in the ascidian *Ciona* has been investigated by Mr D. A. McGill, a Fulbright Scholar, in collaboration with Dr Abbott using the radioisotope ^{131}I; radio-autographs of sectioned material served to indicate the localization in organically bound form within the endostyle.

A preliminary comparison of the mechanical properties of a number of isolated lamellibranch muscles has been made by Dr Abbott in collaboration with Dr Lowy; the results have been published in the *Journal of Physiology*. The muscles used have varied in speed from the fast striated *Pecten* adductor to the very slow posterior adductor in *Mytilus*. It has been shown that when isolated in good condition these muscles are as excitable as vertebrate muscle: they all conform to A. V. Hill's characteristic force-velocity relationship for isotonic shortening and exert isometric tensions of about $2{\cdot}5$ kg./cm.^{2}.

Measurements with a very sensitive piezo electric recorder of the early tension changes after stimulation showed no sign of latency relaxation. Thermal changes during activity are still being studied.

Investigations of the contractile mechanism of lamellibranch muscle has been continued by Dr J. Lowy. The spontaneous electrical and mechanical activity in the smooth and striated parts of *Pecten*'s adductor muscle was recorded simultaneously and continuously for long periods with the intact animal in water. It was found that slow phasic contractions are accompanied by smooth muscle action-potentials whose number and amplitude depend on the extent of contraction and that the maintenance of a state of tension is associated with continuous electrical activity in the smooth muscle. If the nerves leading to this muscle are cut it relaxes and electrical activity ceases completely. The similarity between the contractile mechanism of the *Pecten* smooth muscle and that of the wholly smooth adductors of *Mytilus edulis*, *Mya arenaria* and *Lutraria lutraria* is pointed out, and it is concluded that tonic as well as phasic contraction in smooth lamellibranch muscle is due to nervous excitation.

Observations of spontaneous relaxation and experiments in which relaxation was brought about by reflex stimulation show that the termination of a state of contraction in the smooth *Pecten* muscle is under nervous control of an inhibitory nature. This finding confirms the results of experiments with preparations of the posterior adductor of *Mytilus* and suggests that lamellibranch smooth muscles may be regulated in a manner similar to that found in other invertebrate muscles which do not give a triggered type of response.

On the other hand, it is shown that the *Pecten* striated muscle works by large synchronous contractions and possibly here the elementary reaction is the all-or-none response of whole motor units.

In the light of the results of the comparative observations made with Dr Abbott on isolated lamellibranch muscles, and considering the evidence from experiments with intact lamellibranch adductor muscles, there is now good reason to believe that tonic contraction in lamellibranch smooth muscles is a tetanic phenomenon and that the slow speed of these muscles adequately accounts for their capacity to maintain a state of tension for long periods of time without appreciable fatigue.

An account of the mechanism of contraction and relaxation in the adductor muscle of *Pecten maximus* will appear in the *Journal of Physiology*.

Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

The Library has again been much used by visiting members of the Association.

Published Memoirs

Volume 32, No. 1, of the *Journal* was published in June 1953, and No. 2 in October 1953.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

Atkins, W. R. G. & Jenkins, Pamela G., 1953. Reduction in light accompanying exceptionally heavy rain. *Nature, Lond.*, Vol. 172, p. 79.

Bacci, Guido & La Greca, Marcello, 1953. Genetic and morphological evidence for subspecific differences between Naples and Plymouth populations of *Ophryotrocha puerilis*. *Nature, Lond.*, Vol. 171, p. 1115.

Biebl, Richard, 1953. Über die Seewasserresistenz von Meerstrandpflanzen. *Photographie und Forschung*, Vol. 5, pp. 174–80.

Blaschko, H. & Himms, Jean M., 1954. Enzymic oxidation of amines in decapods. *J. Exp. Biol.*, Vol. 31, pp. 1–7.

Boden, Brian P., 1954. The Euphausiid crustaceans of Southern African Waters. *Trans. Roy. Soc. S. Afr.*, Vol. 34, pp. 181–243.

Brightwell, L. R., 1953. Further notes on the hermit crab *Eupagurus bernhardus* and associated animals. *Proc. Zool. Soc. Lond.*, Vol. 123, pp. 61–64.

Buljan, M., 1951. A modification of Teorell's method for determining small quantities of ammonia. *Arhiv za Kemiju*, Vol. 23, pp. 119–22.

Carlisle, David B., 1953. The larva and adult of *Polycitor crystallinus* Renier (Ascidiacea, Polycitoridae). *Proc. Zool. Soc. Lond.*, Vol. 123, pp. 259–65.

Carlisle, David B., 1953. Note préliminaire sur la structure du système neurosécréteur du pédoncule oculaire de *Lysmata seticaudata* Risso (Crustacea). *C.R. Acad. Sci. Paris*, T. 236, pp. 2541–42.

Carlisle, D. B., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). III. On the activity of the moult-accelerating principle when administered by the oral route (p. 279). IV. On the site of origin of the moult-accelerating principle—experimental evidence (p. 284). *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 278–91.

Carlisle, D. B., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). V. The ovarian inhibiting hormone and the hormonal inhibition of sex-reversal. *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 355–72.

Carlisle, D. B., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). VI. Notes on the structure of the neurosecretory system of the eyestalk. *Pubbl. Staz. Zool. Napoli*, Vol. 24, pp. 434–46.

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Carlisle, D. B. & Dohrn, P. F. R., 1953. Sulla presenza di un ormone d' accrescimento in un crostaceo decapode, la *Lysmata seticaudata* Risso. (On the presence of a growth hormone in a decapod crustacean, *Lysmata seticaudata* Risso). *Ric. sci.*, suppl., 1953, pp. 95–100.

Carlisle, David B. & Knowles, Sir Francis, Bart., 1953. Neurohaemal Organs in Crustaceans. *Nature, Lond.*, Vol. 172, pp. 404–05.

Carlisle, D. B. & Passano, L. M., 1953. The X-organ of Crustacea. *Nature, Lond.*, Vol. 171, pp. 1070–71.

Chapman, Garth, 1953. Studies of the mesogloea of coelenterates. I. Histology and chemical properties. *Q. Journ. Micr. Sci.*, Vol. 94, pp. 155–76.

Chapman, Garth, 1953. Studies on the mesogloea of Coelenterates. II. Physical properties. *J. Exp. Biol.*, Vol. 30, pp. 440–51.

Etherington, D., 1953. On a sporozoon in the coelomic corpuscles of *Phascolosoma minutum* Keferstein (Sipunculoidea). *Parasitology*, Vol. 43, pp. 160–69.

Hart, T. J., 1953. Plankton of the Benguela Current. *Nature, Lond.*, Vol. 171, pp. 631–34.

Hill, M. N. & King, W. B. R., 1953. Seismic prospecting in the English Channel and its geological interpretation. *Quart. J. Geol. Soc. Lond.*, Vol. 109, pp. 1–20.

Holmes, William, 1953. The atrial nervous system of Amphioxus (*Branchiostoma*). *Quart. J. Micr. Sci.*, Vol. 94, pp. 523–535.

James, W. Warwick, 1953. The succession of teeth in elasmobranchs. *Proc. Zool. Soc. Lond.*, Vol. 123, pp. 419–42.

✓ Jones, F. R. Harden, 1951. The swimbladder and the vertical movements of teleostean fishes. *J. Exp. Biol.*, Vol. 28, pp. 553–66.

✓ Kampa, Elizabeth M., 1953. New forms of visual purple from the retinas of certain marine fishes: a re-examination. *J. Physiol.*, Vol. 119, pp. 400–09.

Kerslake, Joy, 1953. Occurrence of *Asparagopsis armata* Harv. on the coast of Devon. *Nature, Lond.*, Vol. 172, p. 874.

Lorch, I. J., Danielli, J. F. & Hörstadius, S., 1953. The effect of enucleation on the development of sea urchin eggs. I. Enucleation of one cell at the 2, 4 or 8 cell stage. *Exp. Cell. Res.*, Vol. 4, pp. 253–62.

Lowenstein, Otto, 1953. Effect of galvanic polarization on the impulse discharge from the horizontal ampulla of the isolated elasmobranch labyrinth. *Nature, Lond.*, Vol. 172, pp. 549–50.

Lowndes, A. G., 1953. The densities of some common Echinodermata from Plymouth. *Ann. Mag. Nat. Hist.*, 12th ser., Vol. 6, pp. 623–24.

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Lowy, J., 1953. Contraction and relaxation in the adductor muscles of *Mytilus edulis*. *J. Physiol.*, Vol. 120, pp. 129–40.

Robertson, James D., 1953. Further studies on ionic regulation in marine invertebrates. *J. Exp. Biol.*, Vol. 30, pp. 277–96.

Russell, Frederick Stratten, 1953. *The Medusae of the British Isles.* Cambridge. (E. T. Browne Monograph of the Marine Biological Association of the United Kingdom.)

Russell, F. S., 1953. The English Channel. *Trans. Devon. Assoc.*, Vol. 85, pp. 1–17.

Silén, Lars, 1954. On the nervous system of *Phoronis*. *Ark. Zool.*, Ser. 2, Bd. 6, pp. 1–40.

Trueman, E. R., 1953. Observations on certain mechanical properties of the ligament of *Pecten*. *J. Exp. Biol.*, Vol. 30, pp. 453–467.

Westblad, Einar, 1953. *Boreohydra simplex* Westblad, a 'bipolar' Hydroid. *Ark. f. Zool.*, Vol. 4, pp. 351–54.

Westblad, Einar, 1953. Marine Macrostomida (Turbellaria) from Scandinavia and England. *Ark. f. Zool.*, Vol. 4, pp. 391–408.

Westblad, Einar, 1953. New Turbellaria parasites in echinoderms. *Ark. f. Zool.*, Vol. 5, pp. 269–88.

Wilson, Douglas P., 1953. Electronic-flash photomicrography. *Discovery*, Vol. 14, pp. 347–349.

Membership of the Association

The total number of members on 31 March 1954 was 770, being 61 more than on 31 March 1953; of these the number of life members was 94 and of annual members 676. The number of Associate members is six.

During the year Prof. Louis Fage has been elected an Honorary Member.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Capital Grant. The Council wish to record their thanks to the Development Commissioners for the very substantial Capital Grant to meet the cost of construction of the new research vessel *Sarsia*. This is the first vessel to be owned by the Association which has been designed and built especially for marine biological research.

Private Income. The Council gratefully acknowledges the following generous grants received during the year:

From the Fishmongers' Company (£300), the Royal Society (£50), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s.*), Leeds (£20), Durham (£10. 10*s.*), Manchester (£10. 10*s.*), Sheffield (£10. 10*s.*), Nottingham (£10. 10*s.*), Southampton (£15. 15*s.*), Exeter (£10. 10*s.*), Leicester (£10. 10*s.*), Hull (£10. 10*s.*), Reading (£21), and the Imperial College of Science and Technology (£10).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1954–55:

President

Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, C.B., C.M.G.
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Prof. A. V. Hill, C.H., O.B.E., Sc.D., F.R.S.
Sir Edward J. Salisbury, Kt., C.B.E., D.Sc., Sec.R.S.
Admiral Sir Aubrey C. H. Smith, K.B.E., C.B., M.V.O.
A. T. A. Dobson, C.B., C.V.O., C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Earl of Verulam

COUNCIL

To retire in 1955

Prof. H. Graham Cannon, Sc.D., F.R.S.
O. D. Hunt
Prof. O. E. Lowenstein, D.Sc.
G. P. Wells, Sc.D.
R. S. Wimpenny

To retire in 1956

J. N. Carruthers, D.Sc.
Prof. H. Munro Fox, F.R.S.
Prof. A. L. Hodgkin, F.R.S.
Prof. J. E. Smith, Sc.D.
Prof. V. C. Wynne-Edwards

To retire in 1957

Miss Anna M. Bidder, Ph.D.
D. J. Crisp, Ph.D.
Prof. J. E. Harris, Ph.D.
C. E. Lucas, D.Sc.
Prof. C. M. Yonge, D.Sc., F.R.S.

Hon. Treasurer

Major E. G. Christie-Miller, 38 Hyde Park Street, London, W. 2

Secretary

F. S. Russell, D.S.C., D.F.C., F.R.S., The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. Wall (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. Christie-Miller
  Harrison S. Edwards
Prof. A. C. Hardy, D.Sc., F.R.S. (Oxford University)
S. Smith, Ph.D. (Cambridge University)
Edward Hindle, Sc.D., F.R.S. (British Association)
H. W. Parker, D.Sc. (Zoological Society)
Prof. A. V. Hill, C.H., O.B.E., Sc.D., F.R.S. (Royal Society)

BALANCE SHEET 1953–54

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 March 1954

			£	£
Capital Reserve Account:				
As at 31st March 1953 ...			111,099	
Add: Grants received and receivable during the year from the Development Fund in respect of cost to date of R.V. 'Sarsia' ...			59,348	170,447
Surplus Account:				
As at 31st March 1953 ...			9,781	
Add: Excess of Income over Expenditure for the year ...			1,703	
			11,484	
Less: Buildings Reconstruction Fund Expenditure transferred ...			32	11,452
				181,899
Aquarium Sinking Fund:				
As at 31st March 1953 ...			279	
Add: Donations for Rebuilding Aquarium Tanks ...			26	
Bank Deposit Interest credited during year ...			2	307
E. T. Browne Bequest Funds:				
		£		
Library Fund as at 31st March 1953 ...		1,260		
Add: Interest on Investments ...		36	1,296	
Special Apparatus Fund as at 31st March 1953 ...		2,714		
Add: Interest on Investments ...		81	2,795	
			4,091	
Less: Scientific Publications Fund as at 31st March 1953		2,290		
Add: Bank Deposit Interest ...		38		
		2,328		
	£			
Less: Payment towards Publication of Monograph on Medusae of the British Isles ...	1,984			
Deficiency on Realization of Investments ...	365	2,349		
			21	4,070
Royal Society Grant—Publication of Monograph on Medusae of the British Isles:				
As at 31st March 1953 ...			766	
Less: Payment to Cambridge University Press ...			2,750	
			1,984	
Less: Paid out of E. T. Browne Bequest—Scientific Publications Fund			1,984	—
A. R. T. Momber Bequest Fund:				
Received during the year ...			938	
Add: Bank Deposit Interest credited ...			4	942
Composition Fees Fund:				
As at 31st March 1953 ...			1,014	
Add: Fees Received during year ...			79	1,093

		£	
Fixed Assets:			
At valuations as estimated by the Director at 31st March 1954:			
Boats and Equipment:			
M.F.V. 'Sula' ...		11,000	
Motor Boat 'Gammarus' ...		200	
		11,200	
Laboratory apparatus, equipment and machinery at cost ...		10,300	
Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost ...		20,200	
R.V. 'Sarsia' at cost ...		137,758	179,458
General Fund Investment at book value			
£352. 2*s.* 3*d.* 2½% Treasury Stock ...			232
(Market Value £228; last year £208)			
E. T. Browne Bequest Fund Investment, at cost			
£4000. 15*s.* 7*d.* British Transport 3% Guaranteed Stock 1978/88 ...			3,912
(Market value £3,441 last year £3,221)			
Composition Fees Fund Investment, at cost:			
£18. 8*s.* 6*d.* 2½% Treasury Stock ...		16	
£1165. 15*s.* 7*d.* British Transport 3% Guaranteed Stock 1978/88 ...		1,077	
(Market value £1,013 last year £874)			1,093
Current Assets:			
Stocks on Hand, as valued by the Director:			
	£		
Specimens ...	600		
Chemicals ...	250		
Journals ...	400		
Nets, gear and general boats' equipment ...	100	1,350	
Sundry Debtors:			
Sales of Specimens, Journals, Nets and Hydrographical Gear	862		
Recoverable Expenditure ...	194	1,056	
Prepayments ...		312	
Balance at Bank and Cash in Hand:			
Coutts & Co., London ...			
Current Account ...	1,812		
Deposit Accounts ...	3,243		
Lloyds Bank Ltd, Plymouth ...	1,766		
Cash in Hand ...	103	6,924	9,642

BUILDINGS RECONSTRUCTION FUND:			
As at 31st March 1953		1,298	
Add: Surplus Account—expenditure transferred		32	
		1,330	
Less: Expenditure during year	8		
Transfer to Radioactive Substances Laboratory Building Fund	186		
		194	1,136
WAR DAMAGE COMPENSATION (PART II):			
Received during year:			
Compensation	764		
Interest (Gross)	240		
		1,004	
Less: Refunded in respect of personal equipment		47	
			957
RADIOACTIVE SUBSTANCES LABORATORY BUILDING FUND:			
Transfered from Buildings Reconstruction Fund		186	
Less: Adverse Balance at 31st March 1953	183		
Expenditure during year	3		
		186	—
RADIOACTIVE SUBSTANCES RESEARCH FUND:			
As at 31st March 1953		584	
Add: Grants Received from the Development Fund		1,360	
		1,944	
Less: Expenditure during year		1,289	
			655
RESEARCH FUND—MISS D. BALLANTINE:			
As at 31st March 1953		87	
Add: Grants Received from the Development Fund		589	
		676	
Less: Expenditure during year		571	
			105
CURRENT LIABILITIES:			
Accrued Expenses		1,408	
Subscriptions and Grants Received in advance		57	
Equipment and Apparatus—R.V. 'Sarsia'		1,708	
			3,173
			£194,337

Note: Capital commitments outstanding amount to £18,072 (1953—£60,670) in respect of the Library extension and New Dogfish store of which £16,365 will be recoverable under a Development Fund Grant

O. D. HUNT
ALASTAIR GRAHAM } *Members of the Council*

£194,337

REPORT OF THE AUDITORS TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1954, and of the excess of income over expenditure for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

PRICE WATERHOUSE & CO.
Chartered Accountants

Norwich Union House
2 St Andrew's Cross
Plymouth
24 *May* 1954

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1954

	£	£
SALARIES, including Pay Additions and Association's contributions to Superannuation Scheme and National Insurance		23,784
LABORATORY AND BOATS' CREWS' WAGES, including Pay Additions, National Insurance, contributions to Superannuation Scheme, War Bonus and Employers' Liability Insurance		19,316
UPKEEP OF LIBRARY		723
SCIENTIFIC PUBLICATIONS, *less* Sales		1,748
UPKEEP OF LABORATORIES AND AQUARIUM:		
Buildings and Machinery	442	
Electricity, Gas, Coal and Water	932	
Chemicals and Apparatus	1,277	
Insurances, Ground Rent and Rent of Stores	164	
Travelling Expenses	670	
Audit Fee	63	
Stationery, Postages, Telephone, Carriage and Sundries	958	
Specimens	195	
		4,701
MAINTENANCE AND HIRE OF BOATS:		
Petrol, Oil, Paraffin, etc.	1,091	
Maintenance of and Repairs to Nets, Gear and Apparatus	2,784	
Boat Hire, Collecting Expenses and Upkeep of Truck	240	
Insurances	1,347	
Hire of 'Decca' Navigator—R.V. 'Sarsia'	166	
Hire of R.V. 'Sabella'	629	
		6,257
ENTERTAINMENT EXPENSES		55
BALANCE, being excess of income over expenditure for the year		1,703
		£58,287

Note: No depreciation is provided on fixed assets.

		£	£
GRANTS AND TABLE RENTS:			
Ministry of Agriculture and Fisheries grant from Development Fund		51,353	
Fishmongers' Company		400	
Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of: London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Southampton £15. 15*s*., Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Reading £21, Sheffield £10. 10*s*., Imperial College £10 and Ministry of Works £104)		1,235	
			52,988
SUBSCRIPTIONS (excluding those received in advance)			648
SALES:			
Specimens		2,438	
Fish		447	
	£		
Nets, Gear and Hydrographical Apparatus	694		
Less: Cost of Materials	351		
		343	
			3,228
INTEREST ON INVESTMENTS			41
INTEREST ON BANK DEPOSITS LESS CHARGES			65
SALE OF DR M. V. LEBOUR'S BOOK			8
SALE OF 'PLYMOUTH MARINE FAUNA'			—
AQUARIUM:			
Admission Fees		1,648	
Sale of Guides		103	
		1,751	
Less: Maintenance of Building	194		
Printing Guides and Tickets	11		
Advertising	51		
Food	83		
Wages	103		
		442	
			1,309
			£58,287

LIST OF GOVERNORS, FOUNDERS, MEMBERS, HONORARY AND ASSOCIATE MEMBERS

1954

GOVERNORS

The British Association for the Advancement of Science, *Burlington House, W.* 1
The University of Oxford
The University of Cambridge
The Worshipful Company of Clothworkers, 48 *Fenchurch Street, E.C.* 3
The Worshipful Company of Fishmongers, *London Bridge, E.C.* 4
 The Prime Warden. (**Council**, 1886→)
 Edwards, Harrison S., *Westhumble Lacey, nr Dorking, Surrey*. (**Council**, 1950→)
 Christie-Miller, Major E. G., 38 *Hyde Park Street, W.* 2. (**Council**, 1941→; **Hon. Treasurer**, 1941→; **Vice-President**, 1951→)
The Zoological Society of London, *Regent's Park, N.W.* 8
The Royal Society, *Burlington House, Piccadilly, W.* 1
Ministry of Agriculture and Fisheries, 3 *Whitehall Place, S.W.* 1
Bayly, Robert (the late). (**Council**, 1896–1901)
Bayly, John (the late)
Browne, E. T. (the late). (**Council**, 1913–19; 1920–37)
Thomasson, J. P. (the late). (**Council**, 1896–1903)
Bidder, G. P., Sc.D. (the late). (**Council**, 1899–1953; **President**, 1939–45; **Vice-President**, 1948–53)
The Lord Moyne, P.C., D.S.O. (the late). (**Vice-President**, 1929; 1939–45; **President**, 1930–39)
Allen, E. J., C.B.E., D.Sc., LL.D., F.R.S. (the late) (Honorary.) (**Council**, 1895–1942; **Secretary**, 1895–1936; **Hon.Governor**, 1937–42)

FOUNDERS

1884 The Corporation of the City of London, *The Guildhall, E.C.* 3
1884 The Worshipful Company of Mercers, *Mercers' Hall*, 4 *Ironmonger Lane, E.C.* 2
1884 The Worshipful Company of Goldsmiths, *Goldsmiths' Hall, Foster Lane, E.C.* 2
1884 The Royal Microscopical Society, *B.M.A. House, Tavistock Square, W.C.* 1
1884 Bulteel, Thos. (the late)
1884 Burdett-Coutts, W. L. A. Bartlett (the late)
1884 Crisp, Sir Frank, Bart. (the late). (**Council**, 1884–92; **Hon. Treasurer**, 1884–88)
1884 Daubeny, Captain Giles A. (the late)
1884 Eddy, J. Ray (the late)
1884 Gassiott, John P. (the late)
1884 Lankester, Sir E. Ray, K.C.B., F.R.S. (the late). (**Hon. Secretary**, 1884–90; **President**, 1891–1929)
1884 Lord Masham (the late)
1884 Moseley, Prof. H. N., F.R.S. (the late). (**Chairman of Council**, 1884–88)

1884 Lord Avebury, F.R.S. (the late). (**Vice-President**, 1884–1913)
1884 Poulton, Prof. Sir Edward B., F.R.S. (the late). (**Council**, 1888–94)
1884 Romanes, Prof. G. J., LL.D., F.R.S. (the late) (**Council**, 1884–91)
1884 Worthington, James (the late)
1885 The 15th Earl of Derby (the late)
1887 Weldon, Prof. W. F. R., F.R.S. (the late). (**Council**, 1890–1901; representing British Association, 1901–5)
1888 Bury, Henry, *The Gate House*, 17 *Alumdale Road*, *Bournemouth West*
1888 The Worshipful Company of Drapers, *Drapers' Hall*, *E.C.* 2
1889 The Worshipful Company of Grocers, *Grocers' Hall*, *Princes Street*, *E.C.* 2
1889 Thompson, Sir Henry, Bart. (the late). (**Vice-President**, 1890–1903)
1889 Lord Revelstoke (the late)
1890 Riches, T. H. (the late). (**Council**, 1920–25)
1892 Browne, Mrs E. T. (the late)
1898 Worth, R. Hansford, M.Inst.C.E. (the late)
1899 The Earl of Iveagh, C.B., C.M.G., 11 *St James's Square*, *S.W.* 1. (**Vice-President**, 1929→)
1902 Gurney, Robert, D.Sc. (the late). (**Council**, 1932–5)
1904 Shaw, Joseph, K.C. (the late)
1909 Harding, Colonel W. (the late)
1910 Murray, Sir John, K.C.B., F.R.S. (the late). (**Council**, 1896–99; **Vice-President**, 1900–13)
1912 Swithinbank, H. (the late)
1913 Shearer, Dr Cresswell, F.R.S. (the late)
1913 Heron-Allen, E., F.R.S. (the late)
1918 Evans, George (the late). (**Hon. Treasurer**, 1915–31; **Vice-President** 1925–33)
1920 McClean, Capt. W. N., 39 *Phillimore Gardens*, *W.* 8
1920 Lord Buckland of Bwlch (the late)
1920 Llewellyn, Sir D. R. (the late)
1921 Harmer, F. W. (the late)
1924 The MacFisheries, Ltd., *Ocean House*, *Pudding Lane*, *E.C.* 3
1924 Lady Murray (the late)
1925 The Institution of Civil Engineers, *Great George Street*, *Westminster*, *S.W.* 1
1925 Discovery Committee
1927 Bidder, Miss Anna M., Ph.D., 4 *Hills Avenue*, *Cambridge* (**Council**, 1948–51, 1954→)
1933 Peel, Col. Sir Edward T., K.B.E., D.S.O., M.C., *c/o Messrs Peel and Co.*, *Ltd.* *P.O. Box* 331, *Alexandria*, *Egypt*. (**Vice-President**, 1936→)
1938 Buchanan, Dr Florence (the late)
1945 Brown, Arthur W. W. (the late)

MEMBERS

* Life Members

1949 Abbott, B. C., Ph.D., A.Inst.P., *The Laboratory*, *Citadel Hill*, *Plymouth*, *Devon*
1945 Aberdeen University Library, *The University*, *Aberdeen*
1934 Adam, Mrs K. M. G., 84 *Lasswade Road*, *Edinburgh* 9
1951 Adams, E., 2 *Woodford Crescent*, *Marsh Mills*, *Plympton*, *Devon*

1940 Adrian, Prof. E. D., O.M., M.D., D.Sc., LL.D., P.R.S., _St Chad's,_ 48 _Grange Road, Cambridge_

1947 Affleck, R. J., 1 _Helmsdale Road, London, S.W._ 16

1949 Aleem, A. A., Ph.D., _Faculty of Science, Farouk I University, Moharram Bey, Alexandria, Egypt_

1950 Alexandrowicz, J. S., Ph.D., M.D., _The Laboratory, Citadel Hill, Plymouth, Devon_

1954 Allen, G. L., _The Nook,_ 87_a Bury Old Road, Sedgley Park, Prestwich, Manchester_

1951 Allen, J. A., _Dept. of Zoology, The University, Glasgow, W._ 2

1952 Allen, Miss J. M., _Tenements Farm, Chipperfield, Herts_

1949 Allen, Mrs M. E., 82 _Oaks Avenue, Worcester Park, Surrey_

1953 Alvariño, Señora A., _Doce de Octubre_ 11–1 °C, _Madrid, Spain_

1927 Amirthalingam, C., Ph.D., 2 _Dickmans Path, Colombo, Ceylon_

1950 Arnold, D. C., _Fair Acre, Abbotskerswell, Newton Abbot, Devon_

1944 Ashby, D. G., 195 _Chesterton Road, Cambridge_

1954 Ashhurst, Miss D. E., _St Hugh's College, Oxford_

*1929 Atkins, Miss D., D.Sc., _The Laboratory, Citadel Hill, Plymouth, Devon_

*1939 Atkins, W. R. G., C.B.E., O.B.E., Sc.D., F.R.I.C., F.Inst.P., F.R.S., _The Old Vicarage, Antony, Torpoint, Cornwall_

*1910 Atkinson, G. T., _Gresham House, Esplanade, Lowestoft, Suffolk_

1951 Atlantic Biological Station, _St Andrews, N.B., Canada_

1948 Baal, H. J., 3 _Bel Royal Villas, Jersey, C.I._

1950 Baerends, Prof. G. P., _Zoölogisch Laboratorium, Rijksstraatweg_ 78, _Haren (Gron.), Holland_

1949 Bagenal, T., _Marine Station, Millport, Isle of Cumbrae, Scotland_

*1952 Baily, Joshua L. Jr., 4435 _Ampudia Street, San Diego_ 3, _California, U.S.A._

1950 Bainbridge, R., Ph.D., 43 _Strathmore Avenue, Hull_

1953 Bainbridge, V., _West African Fisheries Research Institute, Freetown, Sierra Leone_

*1920 Baker, J. R., D.Sc., _Dept. of Zoology and Comparative Anatomy, University Museum, Oxford_

1936 Baldwin, Prof. E., Ph.D., _Dept. of Biochemistry, University College, Gower Street, London, W.C._ 1 (**Council**, 1946–48)

1950 Ballantine, Miss D., _The Laboratory, Citadel Hill, Plymouth, Devon_

1951 Bangor, Marine Biological Station, _University College of North Wales, Bangor, Caern_

1953 Barker, J. A., 27 _Finchley Way, Finchley, London, N._ 3

1949 Barnard, E. E. P., 7 _Webster Gardens, Ealing, London, W._ 5

1939 Barnes, H., Ph.D., F.R.I.C., _Marine Station, Millport, Isle of Cumbrae, Scotland_

1954 Barnes, M. McC., _Mandeville, Rosebank Crescent, Pennsylvania, Exeter, Devon_

1953 Barns, H. N., _Spearwood, Combpyne, near Axminster, Devon_

1939 Barrington, Prof. E. J. W., D.Sc., _Dept. of Zoology, The University, Nottingham_

1951 Barron, H., 65 _Sumerton Road, Belfast, N. Ireland_

1946 Barter, W. Y., 29 _Sea View Avenue, Plymouth, Devon_

1939 Bassindale, R., _Dept. of Zoology, The University, Bristol_

1946 Batham, Miss E. J., Ph.D., _Portobello Marine Biological Station, via Port Chalmers, Otago, New Zealand_

1950 Baughman, J. L., _Las Olas Oceanographic Foundation, Rockport, Texas, U.S.A._

1939 Baxter, E. W., *Biology Dept., Medical School, Guy's Hospital, London, S.E.* 1
*1929 Baylis, L. E., Ph.D., *Dept. of Physiology, University College, Gower Street, London, W.C.* 1
1934 Beadle, L. C., *Dept. of Biology, University College of East Africa, P.O. Box* 262, *Kampala, Uganda*
1928 Beer, Sir Gavin de, Kt., D.Sc., F.R.S., *British Museum* (*Natural History*), *Cromwell Road, London, S.W.* 7
1950 Bell, Mrs E. B., *Solva, Glanford Road, Brigg, Lincs*
1954 Berner, L. D. Jr., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1947 Berrill, Prof. N. J., F.R.S., *Dept. of Zoology, McGill University, Montreal, Canada*
1947 Best, A. C. G., 6 *Station Road, Loudwater, High Wycombe, Bucks*
1948 Betts, Slade, 100 *Avondale Road, Bromley, Kent*
1953 Bhattacharyya, Dr R. N., 44/*B Kalighat Road, Calcutta*-26, *India*
1953 Bhimachar, B. S., D.Sc., F.N.I., *Central Marine Fisheries Research Station, West Hill, Calicut* 5, *South India*
1903 Bidder, Col. H. F., *The Malting House, Nettlebed, near Henley-on-Thames, Oxon*
*1945 Bingley, F. J., *Flatford Mill Field Centre, East Bergholt, near Colchester, Essex*
1925 Birkbeck College, *The Library, Malet Street, London, W.C.* 1
1951 Birkett, L., *Fisheries Laboratory, Lowestoft, Suffolk*
1947 Bishop, M. W. H., *Meadow Farm, Waterbeach, Cambs*
1945 Black, J. A., *Ash House, Caton, near Lancaster, Lancs*
1951 Blackburn, M., *C.S.I.R.O., Fisheries Division, Cronulla, N.S.W., Australia*
1930 Blaschko, Dr H., *Department of Pharmacology, South Parks Road, Oxford*
1952 Blaxter, J. H. S., *Pathside, Frithesden Copse, Berkhamsted, Herts*
1910 Bloomer, H. H., *Longdown, Sunnydale Road, Swanage, Dorset*
1953 Boalch, G. T., *Westfield, Beer, near Seaton, Devon*
1953 Boczarow, B., *Greenbank Hospital, Plymouth, Devon*
1951 Boden, B. P., Ph.D., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1936 Bogue, Prof. J. Yule, D.Sc., *Heyscroft, Hartley Road, Altrincham, Cheshire*
1932 Bolitho, Capt. R. J. B., *Gorey, Jersey, C.I.*
1945 Boney, A. D., 1 *Whiteford Road, Mannamead, Plymouth, Devon*
1954 Bonham, Dr K., *Applied Fisheries Laboratory, University of Washington, Seattle, Washington, U.S.A.*
*1933 Boschma, Prof. Dr H., *Rijksmuseum van Natuurlijke Historie, Leiden, Holland*
1947 Bossanyi, J., *Dove Marine Laboratory, Cullercoats, Northumberland*
1954 Bowers, A. B., *Marine Biological Station, Port Erin, Isle of Man*
*1954 Bradshaw, J. S., *P.O. Box* 891, *Del Mar, California, U.S.A.*
1949 Braithwaite, E. R., *Research Dept,. Acheson Colloids Ltd., Prince Rock, Plymouth, Devon*
1940 Brambell, Prof. F. W. Rogers, D.Sc., F.R.S., *Dept. of Zoology, University College of North Wales, Bangor, Caern.* (**Council** 1944–47, 1948–51)
1954 Brehaut, R. N., *La Canurie, Collings Road, St Peter Port, Guernsey, C.I.*
1924 Brightwell, L. R., 1 *Edith Avenue, Peacehaven, Sussex*
1933 Bristol University, *Dept. of Zoology, Bristol*
*1941 British Celanese Ltd., *Celanese House, Hanover Square, London, W.* 1
1948 British Cod Liver Oils (Hull and Grimsby) Ltd., *P.O. Box No.* 18, *Hull*

1939 British Ropes Ltd., *Western Avenue, Cardiff*
*1946 Brock, Mrs C. H., Ph.D., *Dept. of Agriculture, Downing Street, Cambridge*
1953 Brockman, Miss P. E., *Pathology Dept., South Devon and East Cornwall Hospital, Greenbank Road, Plymouth, Devon*
1946 Brough, Prof. James, D.Sc., *Dept. of Zoology and Comparative Anatomy, University College, Newport Road, Cardiff*
1928 Brown, Miss E. M., Ph.D., 6 *Effingham Lodge, Surbiton Crescent, Kingston-on Thames, Surrey*
1936 Brown, Herbert H., O.B.E., Ph.D., *Fisheries Division, F.A.O. of U.N., Viale delle Terme di Caracalla, Rome, Italy*
*1954 Brull, Prof. L., *Institut de Clinique et de Policlinique Médicales, Hôpital de Baviere, Université de Liége, Liége, Belgium*
1953 Buchsbaum, Prof. R., Ph.D., *Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh* 13, *Pa., U.S.A.*
1953 Buckee, R. E., 32 *Weston Drive, Stanmore, Mddx*
*1925 Bull, Herbert O., D.Sc., *Dove Marine Laboratory, Cullercoats, Northumberland*
1951 Burgess, G. H. O., *Dept. of Zoology, University College, Hull*
1954 Burley, Miss E. A. M., *Avondale*, 2 *Narborough Road South, Leicester*
1948 Burrows, Mrs E. M., Ph.D., *Hartley Botanical Laboratories, The University, Liverpool* 3
1950 Bursa, A., *c/o J. Kordowski*, 206 *W.* 109 *Street, New York* 25, *U.S.A.*
1947 Burton, Miss J. M., *Pinecroft, Ockham Road North, East Horsley, Surrey*
1930 Burton, M., D.Sc., *British Museum (Natural History), Cromwell Road, London, S.W.* 7. (**Council**, 1936–39)
1947 Burton, R. F., *Pinecroft, Ockham Road North, East Horsley, Surrey*
1949 Bush, Prof. S. F., D.Phil., *Dept. of Zoology, University of Natal, Pietermaritzburg, S. Africa*
1949 Butcher, A. W., *Three Salmons Hotel, Usk, Mon*
1950 Butler, Mrs B. R. R., *c/o* 23 *Russell Avenue, Hartley, Plymouth, Devon*

1949 Cameron, H. D., 5 *Compton Park Road, Mannamead, Plymouth, Devon*
*1950 Cameron, Prof. W. M., *Institute of Oceanography, University of British Columbia, Vancouver, Canada*
1920 Cannon, Prof. H. Graham, Sc.D., F.R.S., *Dept. of Zoology, Victoria University, Manchester.* (**Council**, 1927–30, 1932–34, 1937–41, 1942–45, 1952→)
1950 Cant, Miss M. A., 8 *Clyde Road, Sutton, Surrey*
1954 Canterbury Museum, *Rolleston Avenue, Christchurch, New Zealand*
1950 Capstick, C. K., 24 *Kennersdene, Tynemouth, Northumberland*
1953 Carr-Saunders, E. M., *Bridge House, St John's Vale, Keswick, Cumb*
1927 Carruthers, J. N., D.Sc., F.Inst.P., *National Institute of Oceanography, Wormley, near Godalming, Surrey.* (**Council**, 1948–51, 1953→)
1923 Carter, G. S., Ph.D., *Dept. of Zoology, Downing Street, Cambridge*
*1931 Cattell, Dr McKeen, *Cornell University Medical College*, 477 *First Avenue, New York City, U.S.A.*
*1948 Cattley, J. G., *Fisheries Laboratory, Lowestoft, Suffolk*
1949 Centre de Recherches et D'Etudes Océanographiques, 1 *rue Victor Cousin, Paris (Ve), France*
*1949 Chang, H. W., *Zoological Institute, Academia Sinica*, 320 *Yo Yang Road, Shanghai, China*
*1952 Chaplin, Viscount, *Wadstray House, Blackawton, near Totnes, Devon*
*1949 Chapman, Garth, Ph.D., *Nunns, Coxtie Green, Brentwood, Essex*

1951 Chappell, R. J., *Kenilworth, Paul Hill, Newlyn, Penzance, Cornwall*
1936 Charterhouse School, *Biological Dept., Godalming, Surrey*
1947 Cheng, Prof. Chung, Ph.D., *Dept. of Oceanography, National Amoy University, Amoy, China*
*1947 Chidambaram, K., *Fisheries Biological Station, West Hill Post, South Malabar, India*
1952 Childs, P., F.R.C.S., 8 *The Crescent, Plymouth, Devon*
1953 Chile: Dr Parmenio Yañez Andrade, Director, *Marine Biological Station of the University of Chile, Casilla* 13-*D, Viña del Mar, Chile.*
1946 Chipperfield, Philip N. J., Ph.D., *Hillside, Manor Road, Brixham, Devon*
1951 Christie, I. G., 7 *Quarry Park Road, Cheam, Surrey*
1942 Christie-Miller, Major E. G., 38 *Hyde Park Street, London, W.* 2. (**Council**, 1941→; **Hon. Treasurer**, 1941→; **Vice-President**, 1951→)
1952 Clark, J. T., 12 *Hazel Avenue, Torquay, Devon*
1952 Clark, R. B., *Dept. of Zoology, The University, Glasgow, W.* 2
1951 Clarke, Mrs J., 32 *Boolarong Road, Pymble, Sydney, N.S.W., Australia*
1949 Clarke, K. U., *Dept. of Zoology, The University, Nottingham*
1944 Clarke, Robert H., *National Institute of Oceanography, Wormley, near Godalming, Surrey*
1951 Claxton, A. J., 26 *Broad Street, King's Lynn, Norfolk*
1951 Clay, Miss E., *Green Hollow, Gattery Lane, Brixham, Devon*
1936 Clothier, Peter, *Hill Close, Street, Somerset*
1939 Clowes, A. J., *Division of Fisheries, Beach Road, Sea Point, Cape Town, S. Africa*
*1886 Coates and Co. (Plymouth) Ltd., *Black Friars Distillery, Southside Street, Plymouth, Devon*
*1945 Cobham, Lt.-Cdr. A. J., R.N., *Noel Cottage, Castle Street, Porchester, Hants.*
*1925 Cockshott, Lt.-Col. A. M., R.A.S.C., *Cotteswold Naturalists' Field Club, City Library, Gloucester*
1953 Cohen, R. D., 2 *St Lawrence Road, Plymouth, Devon*
1933 Cole, H. A., D.Sc., *Fisheries Laboratory, Burnham-on-Crouch, Essex*
*1948 Collier, Albert, *c/o Fish and Wildlife Service, Ft. Crockett, Galveston, Texas, U.S.A.*
*1885 Collier and Co., 53 *Southside Street, Plymouth, Devon*
1952 Collinge, C. F., 25 *Elmstead Avenue, Chislehurst, Kent*
1954 Collins, D. J., 48 *Belgrave Road, Mutley, Plymouth, Devon*
1950 Collins, William N., 603 *Thatcher Avenue, River Forest, Illinois, U.S.A.*
1947 Collis, Miss M. M., 27 *Mowbray Road, Cambridge*
1930 Colman, J. S., *Marine Biological Station, Port Erin, Isle of Man.* (**Council**, 1951–54)
1947 Cook, Miss P. M., 51 *Runnymeade Crescent, Streatham, London, S.W.* 16
1940 Cook, R. H., *Moor Close, Melbourn, Cambridge*
*1933 Cooper, L. H. N., D.Sc., F.R.I.C., *The Laboratory, Citadel Hill, Plymouth, Devon*
1937 Corbin, P. G., *The Laboratory, Citadel Hill, Plymouth, Devon*
1937 Corbin, Mrs P. G., Ph.D., *Dostabrook, Horrabridge, Devon*
1954 Corcoran, E. F., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1946 Corlett, John, M.Sc., *Fisheries Laboratory, Lowestoft, Suffolk*
1937 Cosway, C. A., *South Devon Technical College, Teignmouth Road, Torquay, Devon*
1941 Cott, H. B., D.Sc., *University Museum of Zoology, Cambridge*

1948 Council for Promotion of Field Studies, *Dale Fort Field Centre, Haverfordwest, Pembs*
1952 Cowper, T. R., *C.S.I.R.O. Fisheries Division, Cronulla, N.S.W., Australia*
1936 Crawford, G. I., 18 *East Drive, Carshalton Beeches, Surrey*
1952 Crawshaw, K. Ridgway, *Heron Cottage, White Notley, Witham, Essex*
1953 Creek, Miss Gwen (Mrs J. M. Grant), 9 *Caergog Terrace, Aberystwyth, Cardigan*
*1928 Crew, Prof. F. A. E., M.D., D.Sc., F.R.S., *Usher Institute, Warrenden Park Road, Edinburgh*, 9
*1952 Crisp, D. J., Ph.D., *Bryn Hydd, Holyhead Road, Bangor, Caern.* (**Council**, 1954→)
1929 Crofts, Miss D. R., D.Sc., *Deerbank, Noisey Wood, Billericay, Essex*
1954 Croghan, P. C., *Dept. of Zoology, Downing Street, Cambridge*
1951 Currie, R. I., *National Institute of Oceanography, Wormley, near Godalming, Surrey*
1954 Curtis, A. S. G., 17 *Parkhill Road, London, N.W.* 3
1950 Cushing, D. H., D.Phil., *Fisheries Laboratory, Lowestoft, Suffolk*
*1930 Cuthbertson, Norman, 101 *Johnstone Avenue, Dartmouth, Nova Scotia, Canada*

1952 Dale, E., 13 *North Street, Royton, Oldham, Lancs*
1922 Dale, Sir Henry H., O.M., G.B.E., F.R.C.P., LL.D., F.R.S., *The Wellcome Trust*, 28 *Portman Square, London, W.* 1. (**Council**, 1922–28)
*1948 Dales, R. Philips, Ph.D., 67 *Westmoreland Avenue, Squirrels Heath, Essex*
1947 Dall, William, *Grace Street, Corinda, S.W.* 4, *Brisbane, Queensland, Australia*
*1919 Damant, Capt. G. C. C., C.B.E., R.N., *Thursford, Cambridge Road, East Cowes, I. of W.* (**Council**, 1928–31, 1937–40)
1939 Danielli, Prof. J. F., D.Sc., *Dept. of Zoology, King's College, Strand, London, W.C.* 2. (**Council**, 1944–45)
1947 Danmarks Akvarium, *Charlottenlund, Denmark.*
1929 Darby, Dr H. H., *Carnegie Institution of Washington*, 5241 *Broad Branch Road, N.W., Washington* 15, *D.C., U.S.A.*
1954 Dare, P. J., 30 *Aylesbury Crescent, Whitleigh, Plymouth, Devon*
1948 Dartmouth: The Royal Naval College, *Dartmouth, Devon*
1952 Davenport, Demorest, Ph.D., *Dept. of Biology, University of California, Santa Barbara College, Santa Barbara, California, U.S.A.*
1920 Davidson, Dr W. Cameron, *Avonleigh, Acadia Road, Torquay, Devon*
1954 Davies, Miss P. A., *Midland Bank House, Fore Street, Kingsbridge, S. Devon*
1931 Dawes, B., D.Sc., *Dept. of Zoology, King's College, Strand, London, W.C.* 2
1944 Day, Prof. J. H., D.F.C., *Dept. of Zoology, University, Rondebosch, Cape Town, S. Africa*
1948 Day, Lionel E., 24 *Inverness Avenue, Westcliff-on-Sea, Essex*
1938 Deacon, G. E. R., C.B.E. D.Sc., F.R.S., *Flitwick House, Milford, Surrey.* (**Council**, 1946–49, 1950–53)
1952 Deevey, Miss Georgiana B., Ph.D., *The Bingham Oceanographic Laboratory, Yale University, New Haven, Conn., U.S.A.*
1952 Delaware: Dept. of Biological Sciences, University of Delaware, *Newark, Delaware, U.S.A.*
1939 Dennell, Ralph, D.Sc., *Dept. of Zoology, The University, Manchester*, 13
1954 Deshpande, R. D., *Zoology Dept., The University, Southampton*

*1915 Dick, G. W., J.P., 500 *Manning Road, Durban, Natal, S. Africa*
1944 Digby, P. S. B., *Dept. of Biology, St Thomas's Hospital Medical School, Westminster, London, S.W.* 1
1948 Dodd, J. M., Ph.D., *Gatty Marine Laboratory, The University, St Andrews, Fife*
1954 Dominion Museum, *Wellington, New Zealand*
1953 Dorsett, D. A., 68 *Slewins Lane, Hornchurch, Essex*
1949 Douglas, Leslie, 11 *Harbour View, Seahouses, Northumberland*
1940 Dowson, Capt. W. B., *Roskellan, Mawnan, near Falmouth, Cornwall*
1954 Dowson, Mrs L. R. M., 27 *Pemberton Gardens, London, N.* 19
1952 Droop, M. R., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1946 Duly, S. J., 68 *Richmond Hill Court, Richmond, Surrey*
1950 Duncan, Miss N., *Royal Holloway College, Englefield Green, Surrey*
1939 Dundee University College Library, *Dundee, Forfar.*
1949 Dussart, B. H., 13 *Quai de Rives, Thonon (Haute-Savoie), France*

*1934 Eales, Miss N. B., D.Sc., *Zoology Dept., The University, Reading*
1945 Edgell, Vice-Admiral Sir John A., K.B.E., C.B., F.R.S., 4 *Royal Avenue, Worcester Park, Surrey.* (**Council**, 1945–48, **Vice-President**, 1948–)
1951 Edwards, C., 45 *Queensbury Park, Rosetta, Belfast, N. Ireland*
1927 Eggleton, P., D.Sc., *Dept. of Physiology, The University, Edinburgh*
1928 Egypt: Coastguard and Fisheries Service, *Alexandria, Egypt*
1948 Elgood, J. H., 27 *Knightscroft Avenue, Rustington, Sussex*
1950 Elliott, Mrs J. H., 39 *Woodhaw, Egham, Surrey*
1953 El Maghraby, A. M., *Queen Mary College, Mile End Road, London, E.* 1
*1929 Elmhirst, L. K., *Dartington Hall, Dartington, near Totnes, Devon*
1951 Etherington, D., 1 *St John's Gardens, London, W.* 11
*1923 Evans, W. Edgar, 38 *Morningside Park, Edinburgh*
1942 Ewer, D. W., *Dept. of Zoology, Natal University College, P.O. Box* 375, *Pietermaritzburg, Natal, S. Africa*

*1929 Faouzi, Dr Hussein, *Faculty of Science (Dept. of Zoology), Farouk I University, Moharram Bey, Alexandria, Egypt*
1953 Farrington, Major G. B. O., 4*b Balmoral Place, The Hoe, Plymouth, Devon*
1948 Faulkner, I. J., Ph.D., *I.C.I. Billingham Division, Billingham, E. Durham*
1950 Felinska, Mrs Maria, Dr Phil., *Diddington, near Buckden, Hunts*
*1933 Fellowes, Miss Rosalind, 23 *The Cloisters, Windsor Castle, Berks*
1950 Fisher, L. R., Ph.D., *Green Bower, Beech Lane, Lower Earley, near Reading, Bucks*
1954 The Fishing News, 68 *Victoria Street, London, S.W.* 1
1928 Ford, E., *Marine Station, Millport, Isle of Cumbrae, Scotland.* (**Council**, 1950–53)
1935 Ford, E. B., D.Sc., F.R.S., *Dept. of Zoology and Comparative Anatomy, University Museum, Oxford*
1953 Ford, D. F., 71 *Brooklyn Road, South Norwood, London, S.E.* 25
1952 Ford, Miss V. E., 43 *Woodbourne Avenue, Brighton, Sussex*
1939 Forrest, J. E., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1950 Forster, G. R., *The Laboratory, Citadel Hill, Plymouth, Devon*
1949 Fountain, H. C., *The Store Cottage, Polbathic, Torpoint, Cornwall.*
1954 Fox, Prof. D. L., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*

1912 Fox, Prof. H. Munro, F.R.S., 2 *Westbourne Terrace Road, London, W.* 2 (**Council**, 1928–30, 1931–34, 1944–47, 1953→)
1942 Foxon, G. E. H., *Dept. of Biology, Guy's Hospital Medical School, London Bridge, London, S.E.* 1
1953 Föyn, Prof. Björn, *Universitetets Biologiske Stasjon, Drøbak, Norway*
1950 Frampton, Cdr R. H. C. F., R.N. (Rtd.), *Ministry of Agriculture and Fisheries, Citadel Hill, Plymouth, Devon.*
1924 Fraser, Miss E. A., D.Sc., *Orchard Cottage, Abinger Common, Dorking, Surrey*
1935 Fraser, F. C., D.Sc., *British Museum* (*Natural History*), *Cromwell Road, London, S.W.* 7. (**Council**, 1950–53)
*1935 Fraser, James H., D.Sc., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1952 Freeman, R. J. H., 43 *Sherwood Road, Croydon, Surrey*
*1939 Fretter, Miss Vera, D.Sc., *Dept. of Zoology, The University, Reading*
1953 Frings, Prof. H., *Dept. of Zoology and Entomology, The Pennsylvania State College, Pennsylvania, U.S.A.*
1949 Fuller, A. S., 30 *Staines Avenue, Cheam, Surrey*
1948 Furness, W. J., *Inglewood, Abbey Park Road, Grimsby, Lincs*

1941 Gardiner, Mrs A. C., *United Nations Korean Reconstruction Agency, P.O. Box* 20, *Grand Central Station, New York*, 17, *N.Y., U.S.A.*
*1928 Gates, Prof. R. R., D.Sc., LL.D., F.R.S., *Biological Laboratories, Harvard University, Cambridge* 38, *Mass., U.S.A.*
1948 Gatty Marine Laboratory (The Principal), *The University, St Andrews, Fife*
1950 Gauld, D. T., Ph.D., *Dept. of Zoology, University College, Achimota, Gold Coast*
1953 Gibbon, Mrs E., *Glenview, Clearbrook, Yelverton, Devon*
1935 Gilson, H. Cary, *Freshwater Biological Association, The Ferry House, Far Sawrey, Ambleside, Westmorland.* (**Council**, 1940–43, 1947–50, 1951–54)
1951 Glaister, Mrs K., 12 *Grey Close, London, N.W.* 11
1945 Glasgow University, *Zoology Dept., Glasgow, W.* 2
1951 Glasson, D. R., Ph.D., 34 *Craven Avenue, St Judes, Plymouth, Devon*
1946 Glover, R. S., *Oceanographical Laboratory*, 78 *Craighall Road, Edinburgh*, 6
1951 Gokhale, S. V., Ph.D., *c/o The Directorate of Marine Products, Jamnagar* (*Saurashtra*), *India*
1951 Gold Coast, University College of, *P.O. Box* 4, *Achimota, Gold Coast*
1954 Goldberg, Dr E. D., *Chemistry Division, Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1951 Goodbody, I. M., *Natural History Dept., Marischal College, Aberdeen*
1954 Goodier, R., *Deanwater Lodge, Woodford, near Bramhall, Cheshire*
1954 Gooding, R. U., *St John's College, Cambridge*
1950 Goodley, E. F. W., 2 *Thickthorn Lane, Laleham, Staines, Mddx*
1939 Goodrich, Dr Helen Pixell, 12 *Park Town, Oxford*
1939 Gordon, Miss Isabella, D.Sc., *British Museum* (*Natural History*), *Cromwell Road, London, S.W.* 7
1943 Gourock Ropework Co., Ltd., 92 *Bay Street, Port Glasgow, Renfrew*
1931 Graham, Prof. Alastair, D.Sc., *Dept. of Zoology, The University, Reading.* (**Council**, 1951–54)
1949 Graham, F. G., *Trethake, Plymstock Road, Oreston, near Plymouth, Devon*
1931 Graham, Michael, C.M.G., O.B.E., *Fisheries Laboratory, Lowestoft, Suffolk.* (**Council**, 1931–32, 1933–36, 1943–46, 1949–52)

1912 Gray, Prof. Sir James, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S., *Dept. of Zoology, Downing Street, Cambridge.* (**Council**, 1920–24, 1928→; representing Cambridge University, 1928–45; **President**, 1945→)
1943 Great Grimsby Coal, Salt and Tanning Co., *Fish Dock Road, Grimsby, Lincs.*
1949 Green, J., 61 *Ruskin Road, Crewe, Cheshire*
1950 Greene, Miss S. M., *Dalegarth, Milner Road, Ansdell, Lytham-St-Annes, Lancs*
1950 Greenfield, Leonard J., *Marine Laboratory, University of Miami, Coral Gables* 34, *Florida, U.S.A.*
*1953 Griffiths, Major R. J., *Rosslyn, Edward Road, Biggin Hill, Kent*
1948 Grigg, Miss Ursula M., *Dove Marine Laboratory, Cullercoats, Northumberland*
1948 Grove, A. V., 86 *Gower Road, Sketty, Swansea, Glam.*
1947 Guiler, E. R., *Dept. of Zoology, University of Tasmania, Hobart, Tasmania*
1947 Gundry, Joseph, and Co., *Bridport, Dorset*
1953 Gunn, Miss B. M., *Merry Ways, Chalk Lane, Ashtead, Surrey*

1951 Hague, Miss K. M., 14 *Ferndale Terrace, Dartmouth, S. Devon*
1946 Haifa: Sea Fisheries Research Station, *P.O. Box* 50, *Haifa, Palestine*
1950 Haines, Surg.-Lieut. D. O., R.N., 217 *Cliffe Road, Strood, Rochester, Kent*
1952 Hall, D. N. F., *Fisheries Research Station, P.O. Box* 749, *Singapore*, 1
1950 Hall, Major H. W., O.B.E., M.C., *Downton Fields, Hordle, Lymington, Hants*
1949 Hall, Miss N. F., *Tyne Brand Products Ltd., North Shields, Northumberland*
1952 Hamburg: Institut für Küsten- und Binnenfischerei, *Neurewall* 72, *Hamburg* 36, *Germany*
*1946 Hamond, Richard, *Morston, Holt, Norfolk*
1954 Hampton, Capt. T. A., *Boat Cottage, Warfleet Creek, Dartmouth, Devon*
1953 Hancock, D. A., *Fisheries Laboratory, Burnham-on-Crouch, Essex*
1954 Hannaford, Miss J. E., 17 *Lorrimore Avenue, Stoke, Plymouth, Devon*
1954 Harding, J. P., Ph.D., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1923 Hardy, Prof. A. C., D.Sc., F.R.S., *Dept. of Zoology and Comparative Anatomy, University Museum, Oxford.* (**Council**, 1938–41, 1942→; representing Oxford University, 1946→)
1950 Harley, Miss M. B., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1932 Harris, Prof. J. E., Ph.D., *Dept. of Zoology, The University, Bristol.* (**Council**, 1946–49, 1950–53, 1954→)
1946 Harris, T. R., 31 *All Saints Road, Wyke Regis, Weymouth, Dorset*
1939 Harrison, R. J., D.Sc., M.R.C.S., L.R.C.P., *Vinicombe, The Woodlands, Farnborough, Kent*
1929 Hart, T. J., D.Sc., *National Institute of Oceanography, Wormley, near Godalming, Surrey*
1934 Hartley, The Rev. P. H. T., *Badingham Rectory, Woodbridge, Suffolk*
1924 Harvey, H. W., Sc.D., F.R.S., *The Laboratory, Citadel Hill, Plymouth, Devon*
1933 Harvey, Prof. L. A., *Dept. of Zoology, University College of the South West, Exeter, Devon.* (**Council**, 1940–43)
1951 Harvey, Mrs M. S. J., *Rowhorne House, Whitestone, Exeter, Devon*
1954 Hasegawa, Yoshio, *Hokkaido Regional Fisheries Research Laboratory, Yoichi, Hokkaido, Japan*
1950 Haswell, G. A., 23 *Russell Avenue, Hartley, Plymouth, Devon*

1954 Hatley, Miss J., 1659 *Pershore Road, Cotteridge, Birmingham*, 30
1952 Hawes, F. A. B., *c/o Grindlay's Bank*, 54 *Parliament Street, London, S.W.* 1
1939 Hayes, Dr F. R., *Dalhousie University, Halifax, N.S., Canada*
1939 Hayes, Mrs F. R., *Dalhousie University, Halifax, N.S., Canada*
1950 Hazevoet, A. C., *Vogelenzangstraat* 19–*I, Amsterdam West, Holland*
1949 Heaysman, Miss J. E. M., 69 *Brook Road, Merstham, Surrey*
1954 Hedgpeth, Dr Joel W., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1948 Hedley, Ronald H., *Remora, Quarry House Lane, Crossgate Moor, Durham City*
1931 Henderson, G. T. D., D.S.C., Ph.D., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh* 6
1953 Henderson, Mrs W. M., 37 *Moor Lane, St Budeaux, Plymouth, Devon*
1925 Hentschel, C. C., 7 *Dudley Court, Upper Berkeley Street, London, W.* 1
1952 Hepper, B. T., *Elmfield, Combe-in-Teignhead, Newton Abbot, Devon*
1950 Herring Industry Board, 1 *Glenfinlas Street, Edinburgh*, 3
1939 Hewer, H. R., *Assistant Professor, Department of Zoology, Imperial College of Science, London, S.W.* 7
1926 Hickling, C. F., C.M.G., Sc.D., *Colonial Office, Sanctuary Buildings, Great Smith Street, London, S.W.* 1. (**Council**, 1947–50)
1951 Hidaka, Prof. K., *Geophysical Institute, Tokyo University, Tokyo, Japan.*
1926 Hill, Prof. A. V., C.H., O.B.E., Sc.D., F.R.S., 16 *Bishopswood Road, Highgate, London, N.* 6. (**Council**, 1925–29, 1930–33, 1934–37, 1938–41, 1942→; representing Royal Society, 1944→; **Vice-President**, 1948→)
*1949 Hill, D. K., *Postgraduate Medical School of London, Ducane Road, London, W.* 12
1947 Hill, M. N., Ph.D., 6 *St Eligius Street, Cambridge*
*1921 Hindle, E., Sc.D., F.R.S., 51 *Warwick Avenue, London, W.* 9. (**Council**, 1946–49; representing British Association, 1950→)
1937 Hinton, M. A. C., F.R.S., *Glaister, Wrington, near Bristol, Somerset*
1926 Hobson, Prof. A. D., *King's College, Newcastle-upon-Tyne* 1, *Northumberland*
1954 Hobson, G. E., 139 *Thornbury Road, Isleworth, Mddx*
1948 Hockley, A. R., *Dept. of Zoology, The University, Southampton*
1939 Hodgkin, Prof. A. L., F.R.S., *Physiological Laboratory, Cambridge* (**Council**, 1951–52, 1953→)
*1953 Hoestlandt, Prof. H., D.Sc., 13 *rue de Toul, Lille (Nord), France*
1952 Holden, M. J., *W.A.F.R.I., Birnin Kebbi, Sokoto Province, N. Nigeria*
1954 Holland: *The Director, Koninklijk Zoologisch Genootschap, Natura Artis Magistra, Amsterdam*
1948 Holme, N. A., *The Laboratory, Citadel Hill, Plymouth, Devon*
1939 Holmes, W., D.Phil., *Dept. of Zoology and Comparative Anatomy, University Museum, Oxford*
1948 Holsgrove, H. E., 121 *Eden Bridge Road, Bush Hill Park, Enfield, Mddx*
1950 Honeker, C., 83 *Marshall Road, Rainham, Kent*
1951 Hornby, Miss C. E., 53 *Langley Drive, Wanstead, London, E.* 11
1933 Horne, F. R., *National Institute of Agricultural Botany, Huntingdon Road, Cambridge*
1950 Horridge, G. A., *St John's College, Cambridge*
1952 Horvath, C., 8649 *Clifton Way, Beverley Hills, California, U.S.A.*
1950 Howard, G. H., 14 *Westlands Avenue, Bargate, Grimsby, Lincs.*
1948 Howe, Surg.-Cdr. (D) D.C., R.N., *H.M.S. 'Terror', R.N. Base, Singapore*
1950 Howells, D. V., 42 *Radstock Road, Reading, Berks*

1953 Howells, Dr W. R., 60 *James Street, Llanelly, Carms*
1932 Howes, N. H., *Dept. of Zoology, University College, Gower Street, London, W.C.* 1
1952 Hoyle, G., *Dept. of Zoology, The University, Glasgow, W.* 2
1953 Hull: The Librarian, *The University College, Hull*
1928 Hunt, O. D., *Corrofell, Newton Ferrers, Devon.* (**Council**, 1944–47, 1948–51, 1952→)
1947 Hunter, W. Russell, *Dept. of Zoology, The University, Glasgow, W.* 2
*1947 Hurrell, H. G., J.P., *Moorgate, Wrangaton, Devon*
1939 Hurst, C. P., *Landulph Rectory, Saltash, Cornwall*
*1920 Hutton, J. Arthur, *Woodlands, Alderley Edge, Manchester*
*1952 Hutton, Robert F., 86 *N. Main Street, Red Lion, Pennsylvania, U.S.A.*

1946 Iceland: *Atvinnudeild Háskólans (Fiskideild), Reykjavik, Iceland*
1945 Imperial Chemical Industries, Ltd., *Nobel House,* 2 *Buckingham Gate, London, S.W.* 1
1954 Indonesia: The Professor of Zoology, *University of Indonesia, Djalan Taman Sari* 64, *Bandung, Java*
1952 Inglis, W. G., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1953 Institute of Seaweed Research, *Inveresk, Midlothian.*

1952 Jackman, L. A. J., 44 *Old Torquay Road, Preston, Paignton, Devon*
1954 Jager, J. C., *van Reesstraat* 122, *The Hague, Holland*
1953 Jagger, J. R., 5 *Duckworth Street, Stoke, Devonport, Devon*
1952 Jean, Prof. Yves, *School of Fisheries, Ste. Anne de la Pocatier, Kam., P.Q., Canada*
1945 Jefferies, H. S., *The Mill, Avoncliffe, Bradford-on-Avon, Wilts*
1952 Jeffery, F. J., *Plymouth College, Plymouth, Devon*
1953 Jellard, Dr C. H., B.M., *Public Health Laboratory, South Devon and East Cornwall Hospital, Greenbank Road, Plymouth, Devon*
1935 Jenkin, Miss P. M., *Dept. of Zoology, The University, Bristol*
1934 Jepps, Miss M. W., D.Sc., 61 *Chesterton Road, Cambridge*
1949 Jeremy, W. H. R., 6 *Elm Grove Road, Exeter, Devon*
1937 Jersey: Conservateur honoraire du Musée de la Société Jersiaise
*1924 Jesus College, *Oxford*
*1947 John, C. C., D.Sc., *Dept. of Marine Biology and Fisheries, Beach P.O., Trivandrum, S. India*
*1947 Johnson, D. S., 10 *St John's Road, Cambridge*
1944 Johnson, Dr F. R., *Osu Fisheries Station, P.O. Box* 630, *Accra, Gold Coast Colony*
*1948 Jones, C. Burdon, Ph.D., *Dept. of Zoology, University College of N. Wales, Bangor, Caern*
*1950 Jones, E. Idris, M.D., 6 *Kewferry Road, Northwood, Mddx*
*1949 Jones, E. W. Knight, Ph.D., *Marine Biological Station, University College of N. Wales, Bangor, Caern*
1949 Jones, F. R. Harden, Ph.D., *Dept. of Zoology, Downing Street, Cambridge*
1952 Jones, T. Howell, *Mydrim House, Llewellyn Street, Tucynon, Aberdare, Glam*
1948 Jones, J. D., *Nantyci, Llysonnen Road, Carmarthen, S. Wales*
1946 Jones, L. W. G., *Cumber House, Manor Road, Brixham, Devon*
1946 Jones, N. S., Ph.D., *Marine Biological Station, Port Erin, Isle of Man*
1953 Jones, P. G. W., *Woodberry, Tolmers Road, Cuffley, Herts*

1949 Jones, R. J., 14 *Canning Road, Croydon, Surrey*
1936 Jones, Rodney R. M., *Tros-yr-Afon, Penmon, Anglesey*
1946 Jones, Prof. R. V., C.B., C.B.E., D.Phil., F.Inst.P., *Dept. of Natural Philosophy, Marischal College, Aberdeen*
1950 Jones, W. C., *Dept. of Zoology, University College of N. Wales, Bangor, Caern.*
1951 Jones, W. E., *Marine Biological Station, University College of N. Wales, Bangor, Caern.*
1947 Jörgensen, C. Barker, *Slettevej* 8, *Copenhagen Söborg, Denmark*
1952 Joysey, Mrs V. C., 26 *Bury Road, Epping, Essex*
1923 Judge, J. J., *Virginia House, Palace Street, Plymouth, Devon*
1950 Juniper, A. J., *The Polytechnic, Regent Street, London, W.* 1

1952 Kabata, Z., 26 *Orchard Road, Aberdeen*
1954 Kain, Miss J. M., 11 *Acol Road, London, N.W.* 6
1951 Kampa, E. M., Ph.D. (Mrs B. P. Boden), *Scripps Institution of Oceanography, La Jolla, Calif., U.S.A.*
1954 Karnatak University: *Karnatak University Library, Dharwar,* (*State Bombay*), *India*
1948 Katterns, L. B., 115 *Feltham Hill Road, Ashford, Mddx*
1945 Katz, Max, Ph.D., *c/o Prof. R. E. Dimick, Dept. of Fish and Game Management, Oregon State College, Corvallis, Oregon, U.S.A.*
1951 Kay, Miss E. A., *Box* 522, *Kolva, Kanai, Territory of Hawaii, U.S.A.*
1940 Keilin, Prof. D., Sc.D., F.R.S., *Molteno Institute, Cambridge.* (**Council**, 1940–43)
1950 Keir, Ronald S., *Newfoundland Fisheries Research Station, Water Street East, St Johns, Newfoundland*
1946 Kelley, D. F., 17 *Bainbridge Avenue, Hartley, Plymouth, Devon*
1954 Kelly, A. L., 352 *Nautilus, La Jolla, California, U.S.A.*
1952 Kemp, Miss J. R., *North Riding Training College, Filey Road, Scarborough, N. Yorks*
1949 Kennedy, G. Y., Ph.D., F.R.I.C., *Dept. of Cancer Research, University Field Laboratory, Blackbrook Road, Sheffield* 10
1946 Kenya: The Game Warden, *Game Department, P.O. Box* 241, *Nairobi*
*1952 Kerkut, G., *Pembroke College, Cambridge*
1951 Kerslake, Miss J., 17 *Hardman Road, Kingston-upon-Thames, Surrey*
*1954 Kesteven, Dr G. L., *c/o Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome, Italy*
1950 Keynes, R. D., Ph.D., *Physiological Laboratory, Cambridge*
1949 Kimmins, B. J., *P.O. Box* 603, *Addis Ababa, Ethopia*
1928 King, Mrs A. Redman, *Mixton House, Lerryn, Lostwithiel, Cornwall*
1947 Kingsbridge Modern Secondary School, *Kingsbridge, Devon*
1952 King's College: *Dept. of Zoology, Newcastle upon Tyne,* 1
1949 Kingston Technical College, *Kingston Hall Road, Kingston-upon-Thames, Surrey*
1954 Kingwell, M. C., *Beechfield, South Brent, S. Devon*
1927 Kirtisinghe, P., *Dept. of Zoology, University of Ceylon, Colombo* 3, *Ceylon*
1950 Kisch, B. S., C.I.E., 38 *Boulevard Thiers, St Jean-de-Luz, B.P., France*
1930 Kitching, J. A., O.B.E., Ph.D., *Dept. of Zoology, The University, Bristol*
1939 Knight, Miss Margery, D.Sc., *University Hall for Woman Students, Holly Road, Fairfield, Liverpool.* (**Council**, 1943–46)
1945 Knowles, Sir Francis G. W., Bt., D.Phil., *Marlborough College, Marlborough, Wilts*

1950 Korn, M. E., 62 *Aberdare Gardens, Hampstead, London, N.W.* 6
1950 Kott, Miss P., *C.S.I.R.O., Fisheries Division, Cronulla, N.S.W., Australia*
1949 Kristensen, Dr Ingvar, *Zoölogisch Station, den Helder, Holland*

1954 Lacey, A. J., 24 *The Gardens, Monkseaton, Northumberland*
1952 Lake, Miss L., *Grove House, Roehampton Lane, London, S.W.* 15
1953 Landa, Antonio, *P.O. Box* 3665, *Balboa, Canal Zone, S. America*
1950 La Rochelle: *Station Océanographique, Pavillon du Port-Le Gabut, La Rochelle (Charente-Maritime), France*
1950 Lasker, Reuben, *Dept. of Biological Sciences, Stanford University, Stanford, California, U.S.A.*
*1925 Lebour, Miss M. V., D.Sc., *Kean Hill, Cawsand, near Plymouth, Devon*
1947 Leehane, J. D. B., 14 *Wyndham Street East, Plymouth, Devon*
1935 LeMare, D. W., *Fisheries Dept., Federation of Malaya and Singapore, Penang, Malaya*
1951 Lewis, J. B., *c/o Dept. of Zoology, McGill University, Montreal, Quebec, Canada*
1952 Lewis, J. R., *Dept. of Zoology, University College of Wales, Aberystwyth, Cardigan*
1951 Lightowlers, F., 109 *Bradford Road, Brighouse, Yorkshire*
1932 Lisbon: Instituto de Biologia Maritima, *Cais do Sodré, Lisbon, Portugal*
1952 Lister-Bradney, J., Ph.D., 25 *Palmerston Road, Westcliff-on-Sea, Essex*
1949 Lodge, Miss S. M., Ph.D., *Marine Biological Station, Port Erin, Isle of Man*
1950 Longhurst, A. R., *Landford Cottage, Landford, Wilts*
1953 Longton, P. A., 37 *Headland Park, North Hill, Plymouth, Devon*
1954 Lorraine, N. S. R., M.D., Ch.B., D.P.H., F.R.S.E., *Public Health Department, London Road, Hadleigh, Essex*
1948 Lovegrove, T., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1953 Lowe, Mrs E. K., *Highfield Lodge, Highfield Road, Carnforth, Lancs*
1950 Lowenstein, Prof. O. E., D.Sc., Ph.D., *Dept. of Zoology, The University, Birmingham*, 15. (**Council**, 1952→)
1926 Lowndes, A. G., Sc.D., F.R.I.C., *c/o The Laboratory, Citadel Hill, Plymouth, Devon*
1950 Lowy, J., Ph.D., *Dept. of Zoology, Queen's University, Belfast, N. Ireland*
1931 Lucas, C. E., D.Sc., *Marine Laboratory, Wood Street, Torry, Aberdeen.* (**Council**, 1949–52, 1954→)
1930 Lumley, Adrian, *Beechcroft, Udney Park Road, Teddington, Mddx*
1953 Lynch, Dr G. A. C., *Beeches, Crapstone, Yelverton, Devon*
1938 Lysaght, Miss A. M., Ph.D., 6 *Cumberland Gardens, London, W.C.* 1

1953 Macaulay, J. H., *c/o John Leckie Ltd.*, 77 *Wellington Street, West Toronto, Ontario, Canada*
1938 MacDonald, R., 112 *Antrim Road, Belfast, N. Ireland*
1935 Mackenzie, Col. W., O.B.E., *c/o Messrs Peel & Co., Ltd., P.O. Box* 331, *Alexandria, Egypt*
1929 Mackinnon, Prof. D. L., D.Sc., 44 *St Leonard's Terrace, Chelsea, London, S.W.* 3. (Council, 1938–42)
1937 Mackintosh, N. A., C.B.E., D.Sc., 7 *Hinde House, Hinde Street, London, W.* 1. (**Council**, 1946–49, 1951–54)
1947 Macnae, William, *Dept. of Zoology, Rhodes University College, Grahamstown, S. Africa*
*1925 Magdalen College, *Oxford*

1951 Malkin, J. J. S., 33 *Elton Parade, Darlington*
1948 Mansfield, A. W., *Dept. of Zoology, McGill University, Quebec, Canada*
1952 Mansfield, Lt.-Col. K. F., *Luckwell Bridge, Wheddon Cross, Minehead, Som*
*1928 Manton, S. M., Sc.D., F.R.S. (Mrs J. P. Harding), 18 *Ennerdale Road, Richmond, Surrey*
1953 Manwaring, Miss R. A., *Dept. of Zoology, University College, Exeter, Devon*
1948 Marcotte, Alexandre, D.Sc., *Marine Biological Station, Grand River, Gaspé Co., P.Q., Canada*
1951 Marshall, C. J., M.D., F.R.C.S., *Penhaven, The Drive, Mevagissey, Cornwall*
1949 Marshall, Douglas, M.P., *Lanwithan House, Lostwithiel, Cornwall*
1951 Marshall, K. E., 126 *Park Road, Peterborough, Northants*
*1947 Marshall, Miss S. M., D.Sc., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1953 Marshall, P. T., *The Priory, Kingswear, Devon*
1951 Mason, J., 270 *Ashton Road East, Hailsworth, Manchester*
1939 Matthews, L. Harrison, Sc.D., F.R.S., *Zoological Society of London, Regent's Park, London, N.W.* 8. (**Council**, 1944–47, 1949–52)
*1947 Matthews, Mrs J. B. (Miss G. C. Evans), *Trevorrick Gate, St Merryn, Cornwall*
1937 Mayne, Cyril F., O.B.E., F.R.C.S., *c/o Barclays Bank Ltd., Plymouth, Devon*
1953 Mayne, R. B., *Martins Meadow, Yelverton, Devon*
1949 McCarthy, Miss D. F., 70 *Halton Terrace, Kensington Park, Adelaide, S. Australia*
1951 McCarton, Miss L., 89 *Eglantine Avenue, Malone Road, Belfast, N. Ireland*
1910 McClean, Capt. W. N., 39 *Phillimore Gardens, London, W.* 8
*1929 McEwen, Mrs Lawrence, *Isle of Muck, Isle of Eigg, Inverness-shire*
*1952 McGill, D. A., *Dept. of Zoology, Columbia University, New York* 27, *New York, U.S.A.*
1954 McGowan, J. A., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1948 McIntyre, A. D., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1950 McIntyre, J. M., *Horsley Hall, Eccleshall, Staffs*
1953 Menker, Donald F., *Marine Laboratory, University of Miami, Coral Gables, Florida, U.S.A.*
*1948 Menon, M. Devidas, *Parvathi Nivas, Diwans Road, Ernakulam, United States of Cochin and Travancore, S. India*
1954 Menzies, Dr R. J., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1953 Mercer, B. V., *Blakewell House, Painswick, Glos*
1953 Meredith, Miss H. M., 7 *The Lynchets, Lewes, Sussex*
1954 Merrett, M. G., 22 *Fullerton Road, Milehouse, Plymouth, Devon*
1950 Messenger, K. G., *Uppingham School, Rutland*
1951 Messent, R. K., 13 *Sedberg Street, Preston, Lancs*
1939 Metropolitan Water Board, 177 *Rosebery Avenue, London, E.C.* 1
1950 Mexico: Department of Physiology, National University, *Apartado Postal* 13699, *Mexico, D.F.*
1947 Miami University Marine Laboratory, *Coral Gables, Florida, U.S.A.*
1951 Mij tot Exploitatie van Kooksovengassen (Mekog), *Ijmuiden, Holland*
1923 Milford Haven Trawler Owners Association Ltd., *Milford Haven, Pembs*

1949 Millar, R. H., Ph.D., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1946 Miller, Cyril J., 42 *Westbourne Road, Peverell, Plymouth, Devon*
1949 Millott, Prof. N., Ph.D., *Dept. of Zoology, University College of West Indies, Mona, Liguanea, Jamaica, B.W.I.*
1953 Mills, D. H., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh* 6
1949 Mistakidis, M. N., *Fisheries Laboratory, Burnham-on-Crouch, Essex*
1947 Mitra, G. N., *Assistant Director of Fisheries, Cuttack (Orissa), India*
1952 Mohamed, Prof. Abdel Fattah, Ph.D., *Oceanography Dept., Faculty of Science, Alexandria University, Maharram Bey, Alexandria, Egypt*
1951 Mohr, J. L., Ph.D., *Dept. of Zoology, University of Southern California, Los Angeles 7, Calif., U.S.A.*
1952 Morgan, Mrs E. M., 11 *Oak Hill Park, Hampstead, London, N.W.* 3
1940 Moore, Hilary B., Ph.D., *Marine Laboratory, Miami University, Coral Gables, Florida, U.S.A.*
1949 Morton, J. E., Ph.D., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1953 Moss, Miss M. P., *Lady Margaret Hall, Oxford*
1931 Mount Desert Island Biological Laboratory, *Salisbury Cove, Maine, U.S.A.*
1938 Mowbray, Louis L., *Curator, Bermuda Government Aquarium, Flatts, Bermuda*
1942 Moynahan, Dr E. J., *Wayside, Jordans, near Beaconsfield, Bucks.*
1953 Muller, R. L., 38 *Brendon Street, London, W.* 1
1952 Munn, R. B., 11002 *East Saragossa Street, Whittier, Calif., U.S.A.*

1952 Naylor, E., *Marine Biological Station, Port Erin, Isle of Man*
1933 Neale, Morley H., C.B.E., *Chaffcombe House, Chard, Somerset.* (**Council** 1934–36, 1939–42, 1943–46, 1947–50; **Vice-President**, 1951→)
1934 Neale and West, *Trawler Owners, Wharf Street, Cardiff*
1939 Needham, Joseph, Sc.D., F.R.S., *Gonville and Caius College, Cambridge*
1947 Newell, G. E., Ph.D., *Dept. of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1953 Newell, B. S., *I.M.F.R.O., P.O. Box* 754, *Zanzibar*
1951 Newfoundland Fisheries Research Station, *Water Street East, St John's, Newfoundland*
1947 Newton, Prof. Lily, Ph.D., *Dept. of Botany, University College of Wales, Aberystwyth, Cardigan.* (**Council**, 1947–50)
1930 Nicholls, A. G., Ph.D., *C.S.I.R.O., Division of Fisheries, Stowell, Hobart, Tasmania*
1950 Nicol, J. A. C., D.Phil., *The Laboratory, Citadel Hill, Plymouth, Devon*
1947 Nisbet, R. H., 8 *Lichfield Road, Woodford Green, Essex*
1948 Nobbs, Miss G. E. D., 4 *Clifton Estate, Plympton, Devon*
1953 Nordli, O., *Statens Fiskarfagskule, Florø, Norway*
1949 Norris, E., *Biology Dept., Kingston Technical College, Kingston Hall Road, Kingston-upon-Thames, Surrey*
1951 North Carolina, University of, *Fisheries Research Laboratory, Morehead City, N. Carolina, U.S.A.*
1950 North, J., 316 *Lee High Road, Lewisham, London, S.E.* 13
1953 North, L. G., 7 *Belsize Square, Hampstead, London, N.W.* 3
1948 Nunn, S. M., *The Laboratory, Citadel Hill, Plymouth, Devon*
1950 Nurse, F. R. (Mrs R. M. Allison), *Biology Dept., Canterbury University College, Christchurch, New Zealand*

1934 Oakley, Prof. C. L., 14 *Cardigan Road, Headingley, Leeds* 6
1954 O'hEocha, C., *Scripps Institution of Oceanography, La Jolla, California, U.S.A.*
1947 Oldfield, Mrs E., *County Technical College, Guildford, Surrey*
1930 Olive, G. W., C.B.E., *Dauntsey's School, West Lavington, near Devizes, Wilts*
1938 Oliver, James H., Ph.D., 16 *Southwark Street, London, S.E.* 1
1953 Oliver, Miss M., *Somerville College, Oxford*
1937 Omer-Cooper, J., *Zoological Dept., Rhodes University College, Grahamstown, S. Africa*
1939 Ommanney, F. D., Ph.D., *Fisheries Research Station, P.O. Box* 749, *Singapore*
1949 Orkin, P. A., *Natural History Dept., The University, Aberdeen*
1947 Orr, A. P., D.Sc., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1945 Otago University Library, *Dunedin, N.I., New Zealand*
*1928 Otter, G. W., *Southwood, Broadstone, Dorset*
1950 Owen, G., *Dept. of Zoology, The University, Glasgow, W.* 2
1953 Owen, Miss G., *Rustington, Flat* 2, *St Wilfred's Road, Worthing*
1949 Owrie, Mrs Harding B., *Dept. of Zoology, Natural Science Building, University of Michigan, Ann Arbor, Mich., U.S.A.*
1951 Oxford: Department of Zoology and Comparative Anatomy, *University Museum, Oxford*

1951 Pacific Biological Station, *Nanaimo, B.C., Canada*
1954 Packham, J. R., *Rylstone*, 8 *Highview Avenue, South Patcham, Brighton*, 6
1953 Pampapathi-Rao, K., Ph.D., *Dept. of Zoology, Erskine College of Natural Sciences, Andhra University, Waltair, India*
*1939 Panikkar, N. K., D.Sc., *Central Marine Fisheries Station, Mandapam Camp P.O., S. India*
*1923 Pantin, C. F. A., Sc.D., F.R.S., *Trinity College, Cambridge.* (**Council**, 1930–32, 1933–36, 1937–40, 1941–44, 1945–49, 1951–53; representing Cambridge University, 1946–49, 1951–53)
1951 Papworth, A. H., *The Terrace, Brixworth, Northampton*
1941 Parke, Miss M. W., D.Sc., *The Laboratory, Citadel Hill, Plymouth, Devon*
1927 Parker, The Hon. John H., *Pound House, Buckland Monachorum, Devon*
1954 Parker, R. H., *Scripps Institution of Oceanography*, 616 *Bonair Street, La Jolla, California, U.S.A.*
1954 Parrish, B. B., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1937 Parry, D. A., Ph.D., *Dept. of Zoology, Downing Street, Cambridge*
1950 Parry, Miss G., *Dept. of Zoology, Downing Street, Cambridge*
*1920 Pass, A. Douglas, *Wooton Fitzpaine, Charmouth, Dorset*
1946 Paul, Mrs Vidya Vati, Ph.D., 116-*D, Kamlanagar, Delhi*-6, *India*
1952 Paul, A. R., 22 *Cooper's Hill, Willingdon, Eastbourne, Sussex*
1925 Pawlyn Bros., *Mevagissey, Cornwall*
1925 Pawlyn, T. A., *Mevagissey, Cornwall*
1933 Peel, Col. Sir Edward T., K.B.E., D.S.O., M.C., *c/o Messrs Peel & Co., Ltd., P.O. Box* 331, *Alexandria, Egypt.* (**Vice-President**, 1936→)
1948 Peiping, Institute of Zoology, National Academy of Peiping, 58 *Museum Road, Peiping* 7, *China*
1953 Pelly, Miss J., *Lady Margaret Hall, Oxford*
1939 Pennel, V., 28 *Huntingdon Road, Cambridge*
1925 Pentelow, F. T. K., *Old Slade Lane, Iver, Bucks*
1929 Percival, Prof. E., *Canterbury University College, Christchurch, New Zealand*

1947 Perkins, E. J., *Cross Keys, Post Office, near Hereford*
1936 Perkins, F., *Red Hand Compositions Co.*, 2 *The Firs, York Road, Ripon, Yorks*
1951 Perry, Lt.-Cdr. W. J., U.S.N., *Office of Naval Research*, 34 *Grosvenor Square, London, W.* 1
1950 Phillipson, J., Ph.D., *Dept. of Zoology, Science Laboratories, South Road, Durham*
1934 Picken, L. E. R., Ph.D., *Trinity College, Cambridge*
1940 Pike, R. B., Ph.D., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1953 Pittam, M. D., *Magdalen College, Oxford*
1906 Plymouth Corporation (Museum Committee), *Tavistock Road, Plymouth, Devon*
1910 Plymouth Education Authority, *Cobourg Street, Plymouth, Devon*
1906 Plymouth, Port of, Incorporated Chamber of Commerce, *Plymouth, Devon*
1924 Plymouth Proprietary Library, 1 *Alton Terrace, North Hill, Plymouth, Devon*
1929 Plymouth Public Library, *Tavistock Road, Plymouth, Devon*
1948 Pollard, D. G., 60 *Alcester Street, Stoke, Plymouth, Devon*
1950 Polytechnic, *The Library, Regent Street, London, W.* 1
1947 Portsmouth Grammar School, *Biology Department, Portsmouth, Hants*
1929 Portsmouth Municipal College, *Portsmouth, Hants*
1950 Posner, Gerald S., *The Bingham Oceanographic Laboratory, Box* 2025, *Yale Station, New Haven, Conn., U.S.A.*
1953 Pounce, A. G., *Laurel Villa, Meopham, near Gravesend, Kent*
1950 Powell, H. T., *Marine Station, Millport, Isle of Cumbrae, Scotland*
1950 Powell, R. R., 170 *Streatfield Road, Kenton, Mddx*
1954 Pratt, Dr Ivan, *Dept. of Zoology, Oregon State College, Corvallis, Oregon, U.S.A.*
1951 Pringle, I. G., St C., *Preston Hall, Uppingham, Rutland*
1939 Pringle, J. W. S., M.B.E., F.R.S., *Dept. of Zoology, Downing Street, Cambridge*
1954 Pritchard, Dr D. W., *Chesapeak Bay Institute, The Johns Hopkins University, Box 426A, RFD 2, Annapolis, Maryland, U.S.A.*
*1947 Pryor, Thomas, D.S.O., *Westbourne House, Saltash, Cornwall*
1939 Pumphrey, Prof. R. J., Ph.D., F.R.S., *Dept. of Zoology, The University, Liverpool*
*1939 Purchon, Prof. R. D., Ph.D., *Dept. of Zoology, University of Malaya, Singapore* 10

1953 Quebec: Station de Biologie Marine, *Grande Rivière, Gaspé, P.Q., Canada*
1936 Queen Mary College, *Dept. of Zoology, Mile End Road, London, E.* 1
1954 Queensland Museum, *Brisbane, Queensland, Australia*
1939 Queen's University, *Belfast, N. Ireland*
1947 Queckett Microscopical Club (W. Burrells, Hon. Sec.), 52 *Draycott Avenue, Northwick Park, Harrow, Mddx*
1932 Quick, H. E., F.R.C.S., *Craythorne, Shinfield Road, Reading, Berks*
1952 Quiroga, Dr M., *Constitucion* 36, *Veracruz, Ver., Mexico*

1954 Radford, Miss M., 27 *Thornhill Road, Mannamead, Plymouth, Devon*
1932 Ramalho, Dr A., *Instituto de Biologia Maritima, Cais do Sodré, Lisbon, Portugal*
1949 Rasmussen, E., *The Zoological Laboratory, Landbohøjskolen, Bülowsvej, Copenhagen V, Denmark*
1939 Rawlinson, Miss Ruth, 23*a Holbrook Road, Stoneygate, Leicester*

1947 Raymont, Prof. J. E. G., *Dept. of Zoology, The University, Southampton*

*1933 Rayner, G. W., *c/o C.S.I.R.O., Fisheries Division, Cronulla, N.S.W., Australia*

1949 Rees, C. B., D.Sc., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh* 6

*1936 Rees, W. J., D.Sc., 37 *Kings Road, Richmond, Surrey*

1951 Reish, Donald J., *Allan Hancock Foundation, University of Southern California, Los Angeles, California, U.S.A.*

1935 Renouf, Prof. L. P. W., *University College, Cork, Eire*

1953 Research Organization of Ships' Compositions Manufacturers, Ltd., *Grosvenor Gardens House, Grosvenor Gardens, London, S.W.* 1

1940 Richards, P. B., *c/o Lloyds Bank Ltd., Truro, Cornwall*

1951 Rickard, Miss B., 68 *Furze Hill Road, Mutley, Plymouth, Devon*

1946 Rifaat, Ahmed, *Alexandria Institute of Hydrobiology and Fisheries, Kayed Bey, Alexandria, Egypt*

1951 Rigby, Miss J. E., 184 *Walm Lane, London, N.W.* 2

1952 Rijal, Z., *c/o Government Boys' Secondary School, P.O. Box* 873, *Zanzibar*

1953 Riksmuseum (The Director), *Evertebratavd., Stockholm, Sweden*

*1919 Ritchie, Prof. A. D., *Old College, The University, Edinburgh.* (**Council**, 1929–32, 1934–37)

1944 Ritchie, Prof. James, C.B.E., D.Sc., 31 *Mortonhall Road, Edinburgh.* (**Council**, 1944–47)

1953 Robertson, J. D., Ph.D., F.R.S.E., *Zoology Dept., The University, Glasgow, W.*2

1948 Robinson, G. A., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh* 6

1949 Rodhe, Dr W., *Institute of Limnology, Uppsala, Sweden*

1940 Rogers, Dr T. Howard, *Naval Research Establishment, Halifax, Nova Scotia, Canada*

1952 Rosa, Horatio, *F.A.O. of the United Nations, Western South American Regional Office, Huérfanos* 786–7*th floor, P.O. Box No.* 10095, *Santiago de Chile*

1939 Ross, D. M., Ph.D., *Dept. of Zoology, University College, Gower Street, London, W.C.* 1

1939 Ross, Miss Sybil S., 158 *Craiglea Drive, Edinburgh* 10

*1931 Rothschild, Rt Hon. Lord, G.M., Ph.D., F.R.S., *Merton Hall, Cambridge.* (**Council**, 1939–42)

*1932 Rothschild, The Hon. Miriam (The Hon. Mrs George Lane), *The Cottage, Ashton Wold, Peterborough, Northants.* (**Council**, 1942–45)

1951 Rowe, Rev. A. F. C., *The Vicarage, Kingskerswell, Newton Abbot, Devon*

1945 Rowett, Miss H. G. Q., *Plymouth Technical College, Tavistock Road, Plymouth, Devon*

1952 Royal Holloway College, *Englefield Green, Surrey*

1924 Rudge, Chas. H., *Trevalgan, Feliskirk Lane, Marazion, Cornwall*

1927 Russell, F. S., D.S.C., D.F.C., F.R.S., *The Laboratory, Citadel Hill, Plymouth, Devon.* (**Council**, 1945→; **Secretary**, 1945→)

1951 Russell, S. M. M., *Dept. of Zoology, Birkbeck College, Malet Street, London, W.C.* 1

1954 Ryland, J. S., 182 *Manor Road North, Thames Ditton, Surrey*

1946 Sagay, A. A. E., *Warri College, Warri, Nigeria*

1927 St Aubyn, Capt. The Hon. Lionel, M.V.O., *Saltram, Plympton, Devon*

1953 St Aubrey, Miss S. N. D., *St Martin's Vicarage, Vicar's Road, Gospel Oak, London, N.W.* 5

*1951 Salisbury, A. E., *Ormonde House*, 51 *Amersham Hills, High Wycombe, Bucks*
*1934 Salisbury, Sir Edward J., Kt., C.B.E., D.Sc., Sec.R.S., 49 *Kew Green, Kew Gardens, Surrey.* (**Council**, 1934–37, 1940–43; **Vice-President**, 1948→)
1950 Samuel, J. H., 68 *Conway Road, Cardiff*
1946 Sartory, P. K., 107 *Rivermeads Avenue, Twickenham, Mddx*
1911 Saunders, J. T., *University College, Ibadan, Nigeria*
1914 Savage, R. E., O.B.E., *Monfa, Pentywyn Road, Deganwy, Caern*
1946 Saville, Alan, *Marine Laboratory, Wood Street, Torry, Aberdeen*
*1945 Saville, Tom C., 31 *Mawton Crescent, Wollaton Park, Nottingham*
*1921 Scott, Peter M., M.B.E., D.S.C., *New Grounds, Slimbridge, Glos*
1953 Scott, A., 2 *North Street, Strichen, Aberdeenshire*
1953 Seaford College, *Larrington Park, Petworth, Sussex*
1950 Segrove, F., Ph.D., *Dept. of Zoology, The University, Sheffield*, 10
1928 Sen, B., *Vivekanada Laboratory, Baghbazar, Calcutta, India*
1922 Sewell, Lt.-Col. R. B. Seymour, C.I.E., Sc.D., F.R.S., *Dept. of Zoology, Downing Street, Cambridge.* (**Council**, 1935–38)
1950 Sharman, D. P., 69 *Wentworth Road, Harborne, Birmingham*, 17
1953 Sharman, Mrs D. P., 69 *Wentworth Road, Harborne, Birmingham*, 17
1947 Sharman, Miss M., 42 *Winchester Drive, Wallasey, Cheshire*
1953 Shaw, G. W., *West Buckland School, Barnstaple, N. Devon*
1933 Sheffield, *The Library, The University, Sheffield* 10
1952 Shelbourne, J. E., *Fisheries Laboratory, Lowestoft, Suffolk*
1953 Sherbourne, D. H., 85 *Harrowdene Road, Wembley, Mddx*
1935 Siam: Department of Fisheries, *Bangkok, Siam*
1940 Simpson, A. C., *Fisheries Laboratory, Lowestoft, Suffolk*
1953 Sims, Rudolph W., Jr., *Marine Laboratory, Box* 488, *Coral Gables* 46, *Florida, U.S.A.*
1946 Sinclair, Horace, *Southsea Aquarium, South Parade, Southsea, Hants*
1954 Skinner, J. G. 112 *Gurney Court Road, St Albans, Herts*
*1884 Skinners, The Worshipful Company of, *Skinners' Hall, Dowgate Hill, London, E.C.* 4
1948 Slack, H. D., Ph.D., *Dept. of Zoology, The University, Glasgow, W.* 2
1952 Slinn, D. J., *Marine Biological Station, Port Erin, Isle of Man*
1953 Smaridge, Mrs M. W., 116 *Pine Gardens, Eastcote, Ruislip, Mddx*
1949 Smith, Major A. A. Dorrien, D.S.O., *Tresco Abbey, Isles of Scilly, Cornwall*
1948 Smith, Admiral Sir Aubrey, K.B.E., C.B., M.V.O., *Hay's Wharf and Dock, Southwark, London, S.E.* 1. (**Council**, representing Fishmongers' Company, 1937–46, **Vice-President**, 1948→)
1932 Smith, B. Webster, 87 *Northumberland Road, New Barnet, Herts*
1949 Smith, F. F., *Brentwood, Fords Avenue, Healing, Lincs*
1929 Smith, F. G. W., *Zoology Dept., Miami University, Coral Gables* 34, *Florida, U.S.A.*
1932 Smith, Prof. J. E., Sc.D., 149 *Huntingdon Road, Cambridge.* (**Council**, 1943–46, 1948–51, 1953→; representing Cambridge University, 1949–51)
1954 Smith, R. I., Ph.D., *Dept. of Zoology, University of California, Berkeley* 4, *Calif., U.S.A.*
1939 Smith, S., Ph.D., *St Catharine's College, Cambridge.* (**Council**, representing Cambridge University, 1953→)
1953 Snell, R. J., 63 *Shaldon Drive, South Ruislip, Mddx*
1929 South Africa, Fisheries Division of, *Beach Road, Sea Point, Cape Town, S. Africa*

1943 Southampton, The University
1945 Sparrow, B. W., *Corrofell, Newton Ferrers, Devon*
1934 Spaul, Prof. E. A., D.Sc., Ph.D., *Dept. of Zoology, The University, Leeds*
1951 Spencer, C. P., Ph.D., *Marine Biological Station, University College of N. Wales, Bangor, Caern.*
1930 Spooner, G. M., M.B.E., *The Laboratory, Citadel Hill, Plymouth, Devon*
1938 Spooner, Mrs G. M., Ph.D., *Fiveoaks, Crapstone, Yelverton, Devon*
*1939 Sproston, Miss Nora G., *c/o The Director, Zoological Survey of India, Japakusum House,* 34 *Chittarajan Avenue, Calcutta* 12, *India*
1939 Stanbury, Miss F. A., *Biology Dept., Plymouth Technical College, Tavistock Road, Plymouth, Devon*
1954 Stanley, T. P. G., *Thorn, Wembury, near Plymouth, Devon*
1953 Steel, J. H., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1925 Stephen, A. C., D.Sc., *Royal Scottish Museum, Edinburgh*
1951 Stephenson, R. M., *Somewhere, St Mary's, Isles of Scilly, Cornwall*
1930 Stephenson, Prof. T. A., D.Sc., F.R.S., *Dept. of Zoology, University College of Wales, Cambrian Street, Aberystwyth, Cardigan.* (**Council**, 1941–44)
*1949 Stephenson, W., Ph.D., *Dept. of Zoology, University of Queensland, Brisbane, Australia*
1951 Steven, D. H., *Willowby House, Yelverton, Devon*
1950 Steven, G. A., D.Sc., F.R.S.E., *Willowby House, Yelverton, Devon*
1930 Steven, Mrs G. A., *Willowby House, Yelverton, Devon*
1935 Stewart-Evison, Chas., *Manor East Lodge, Sidbury, Sidmouth, Devon*
1947 Stobart, T. R., *Countryman Films Ltd.,* 18 *Soho Square, London, W.* 1
1952 Stock, W. O., 24 *Hanham Road, Kingswood, near Bristol*
*1934 Stoneman, Eric, *Zoology Dept., The University, Reading*
1950 Street, P. A. R., 6 *Bellfield Avenue, Harrow Weald, Mddx*
1936 Stubbings, H. G., Ph.D., 13 *Third Avenue, Denvilles, Havant, Hants*
1935 Sturdy, Mrs R. S., *Woodpiece, Apsley Guise, Bletchley, Bucks*
1946 Subrahmanyan, R., Ph.D., 52 *Choolai High Road, Madras* 7, *India*
1952 Surtees, S. R., *Ministry of Agriculture, Infestation Control Division,* 29 *Rodney Street, Liverpool,* 1
1950 Sutcliffe, W. H., Jr., Ph.D., *Bermuda Biological Station, St George's West, Bermuda*
1949 Sutton, Miss M. F., Ph.D., 6 *Effingham Lodge, Surbiton Crescent, Kingston-on-Thames, Surrey*
1952 Symons, H. W., *The Old Forge, Cowbeach, Hailsham, Sussex*

1948 Tait, R. V., 62 *Nightingale Road, Rickmansworth, Herts*
1953 Talbot, F. H., *East African Marine Fisheries Research Organization, P.O. Box* 668, *Zanzibar*
1932 Taylor, Prof. Sir Geoffrey I., D.Sc., F.R.S., *Farmfield, Huntingdon Road, Cambridge.* (**Council**, 1932–35)
*1922 Taylor, Joseph A., 22 *Eaton Place, Brighton* 7, *Sussex*
1954 Tebble, N., *British Museum (Natural History), Cromwell Road, London, S.W.*7
1942 Templeman, Dr Wilfred, Director, *Newfoundland Fisheries Research Station, St John's, Newfoundland*
1948 Tham, Ah Kow, *c/o Fisheries Dept.,* 4*th Floor, Fullerton Building, Singapore*
1953 Thomas, H. Wilson, M.R.C.S., L.R.C.P., *Furzehatt, Plymstock, near Plymouth, Devon*
1939 Thomas, I. M., *Dept. of Zoology, The University, Adelaide, S. Australia*

1950 Thomas, Miss J. G., Ph.D., *Royal Holloway College, Englefield Green, Surrey*
1947 Thompson, J. A., *c/o G. & F. Henderson, Oathill Farm, Enstone, Oxon*
1952 Thorson, Gunnar, D.Phil. *Universitetets Zoologiske Museum, Krystalgade, Copenhagen K, Denmark*
1953 Tobias, Miss M. A., 4*a Allan Way, North Acton, London, W.* 3
1903 Torquay Natural History Society, *The Museum, Torquay, Devon*
1939 Totton, A. K., M.C., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1936 Tremblay, Jean-Louis, D.Sc., *Station Biologique du Saint Laurent, Faculté des Sciences, Boulevard de l'Entente, Quebec, Canada*
*1946 Tripathi, Y. R., *c/o S. R. Tripathi, Esq., Narsinghgarh, C.I., India*
1938 Trout, Geoffrey C., *Fisheries Laboratory, Lowestoft, Suffolk*
1936 Truelove, P. H., F.R.I.C., *Red Hand Compositions Co.,* 2 *The Firs, York Road, Ripon, Yorks*
1946 Trumble, Prof. H. C., *Waite Institute, Private Mail Bag, Adelaide, S. Australia*
1948 Trythall, Major E. R., *Houndiscombe Place,* 60 *North Road, Plymouth, Devon*
1947 Tuck, A. W., *Upriver, Potter Heigham, Norfolk*
1943 Tucker, Denys W., *British Museum (Natural History), Cromwell Road, London, S.W.* 7
1954 Tucker, H. S., *Roseland, Menheniot, Liskeard, Cornwall*
1939 Tutin, Prof. T. G., *Botany Dept., University College, Leicester*

1950 Umpelby, G., 1 *Togo Terrace, The Hoe, Plymouth, Devon*
1951 Upton, Miss B. C., 21 *Buxton Avenue, Caversham, Reading, Berks*

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